Pharmacological and Genetic Effects of Serotonin on Value-Based Decision-Making

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Meiner Familie,

die, die da ist und die da kommen wird

Vorwort

Die vorliegende Doktorarbeit wurde publikationsbasiert, jedoch gemäß § 8 Absatz 1 der Promotionsordnung vom 23.02.2011 dem Bereich Mathematik und Naturwissenschaften an der Technischen Universität Dresden als abgeschlossene Einzelarbeit, verfasst. Sie umfasst zwei Artikel, die bereits in Fachzeitschriften veröffentlicht worden sind und ein Manuskript, das zur Publikation vorgesehen ist. Alle Fachartikel entstanden im Rahmen des Teilprojektes B04 „Einfluss von Serotonin auf Entscheidungs- und Lernvorgänge“ des Sonderforschungsbereiches 940 „Volition und Kognitive Kontrolle“ an der Technischen Universität Dresden. Die Kapitel 2 – 4 enthalten die genannten Publikationen und das Manuskript:

Kapitel 2 (Studie 1)

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Kapitel 3 (Studie 2)

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Kapitel 4 (Studie 3)

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Exklusivität der Publikationen

Diese Publikationen werden aktuell nicht in anderen Dissertationen verwendet und sind nach aktuellem Stand dafür auch zukünftig nicht vorgesehen.

Unterschrift Promovend Unterschrift Betreuer

Die Doktorarbeit wurde in englischer Sprache verfasst, um die Einheit zu den Fachartikeln zu gewährleisten.

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Abstract

Serotonin (5-HT) has been evidenced in reward and punishment processing during decision-making processes while sophisticated computational theories, similar to dopamine, have been scarce. A recent (2011) computational framework provided by Cools, Nakamura, and Daw has made a strong effort to address this issue, providing an elegant mechanism through which the role of 5-HT on decision-making behaviour across different choice domains can purportedly be predicted. Therefore, the main aim of this thesis was to comprehensively test the predictions of this framework using four different tasks from our value-based decision-making battery and an acute tryptophan intervention in a double-blind sham-controlled cross-over design to manipulate central tonic 5-HT levels together with magnetic resonance imaging. Furthermore, the role of the *5-HTTLPR*, which has been shown to be an important marker of 5-HT functioning, was also investigated.

In the first study (Chapter 2) on the effect of tonic 5-HT levels and *5-HTTLPR* on probabilistic choice was focused upon. While no significant effect of our intervention on behaviour was determined, a significant association between *5-HTTLPR* and risk-seeking for losses was found, indicating that S/S carriers had the highest scores, while L/L carriers possessed the lowest.

The second study (Chapter 3) investigated the role of tonic 5-HT levels *5-HTTLPR* on intertemporal choice, both on the behavioural and brain level. Here, no significant effect of either the intervention, or genotype on behaviour and the brain was uncovered.

Finally, in study 3 (Chapter 4), we assessed whether the genetic association between *5-HTTLPR* and risk-seeking for losses might be explained via differences in white matter microstructure as measured with diffusion-weighted imaging (DWI). No relationship between the DWI parameters and risk-seeking for losses, as well as no linear relationship between the genotype and the DWI parameters as expected by our findings from chapter 2 were found.

Overall, the findings presented in this thesis suggest that the influence of 5-HT on decision-making is not strong according to how we measured it. Nevertheless, this thesis' findings highlight and discuss important gaps between human and nonhuman animal research. Addressing these issues in the future will improve the understanding of the 5-HT system both on a theoretical and experimental level.

1. General Introduction

1.1. Decision-making and moral philosophy

Research on human decision-making has a short history, but a long past. Reason, desire, and spirit as drivers of decisions can be traced to Ancient Greek philosophy, one prominent example being found in Plato's *Republic* (2005). In his seminal work, he describes conversations in which Socrates envisions a prosperous city ruled by members of a guardian class, who are the most dedicated to the city's needs (412c-e). Importantly, not everyone can become a guardian, only those with a balanced soul in which reason and spirit control desire (441e, 442a) allowing for decisions to be made, which are beneficial to the guardians as well as the rest of the citizens. Furthermore, this fictional city is ruled so that it becomes neither too wealthy nor too poor (421e), as 'one produces luxury, idleness, and revolution, the other meanness of spirit and poor workmanship – and of course revolution as well.' (422a). Plato was perhaps the first to articulate two aspects of decision-making that remain highly relevant in the present: understanding the mechanisms that drive decision-making (reason and desire) and what constitutes a good decision (beneficial outcome).

These aspects have also played a major role in the normative ethics of moral philosophy, such as utilitarianism (i.e. Bentham, 1789). His timeless work, '*An Introduction to the Principles of Morals and Legislation'* begins with the sentence 'Nature has placed mankind under the governance of two sovereign masters, pain and pleasure' (p. 6)[1]. According to Bentham's definition of utilitarianism, human action should be guided by the '*principle of utility'*, which states that pleasures (i.e. happiness, benefit, and good) should be pursued while pains (i.e. evil, mischief, and unhappiness) minimised or prevented. Following these rules, any action is evaluated solely on its consequences (cf. Nasher, 2009, p. 14 ff) assuming that it most likely brings the greatest possible happiness. Interestingly, as actions are only judged by their outcomes, the personal motives of the agent are seen as irrelevant. This philosophic approach sidesteps the need to measure unobservable psychological processes that play a role in the decision-making process, while focusing on the outcome (consequence) of an action. Interestingly, Bentham suggests measuring the value of a pleasure on the basis of parameters such as intensity, duration, (un)certainty and nearness/remoteness (Bentham, 1789, p. 22).

[1] The page number refers to the book format as it is published on earlymoderntexts.com

While the concepts of utilitarianism describe a norm under which a society flourishes if its members follow their ethics (e.g. principle of utility) it remains vague with respect to the economic point of view, contrasting with Plato for example, who established rules and structures for a prosperous society with a stronger emphasis on societal classes (i.e. the Guardians) and early, greatly prescient ideas about economics[2].

One of Bentham's contemporaries – the moral philosopher Adam Smith, connected these two streams, penning two works, which remain significant to this day: *The Theory of Moral Sentiments*, published in 1759 and *An Inquiry into the Nature and Causes of the Wealth of Nations* (known most famously, albeit erroneously, by the last three words of its title), published in 1776. The first of these concerns what constitutes human nature, how moral judgements are made, and how mankind interacts in a way that permits peaceful coexistence. The second, more well-known publication discusses the division of labour, accumulation of capital, benefits of free markets, thoughts on the role of governments and taxes and, interestingly, how human nature is perfectly shaped for such a system. Smith understood that human beings mainly act out of interest for self-preservation, rather than the interests of others (Smith, 1759, part VI, ch. 1, p. 116, para 1)[3], all the while continuing to feel sympathy for others. This is a very strong theme in *The Theory of Moral Sentiments*, which begins with the following, 'No matter how selfish you think man is, it's obvious that there are some principles in his nature that give him an interest in the welfare of others, and make their happiness necessary to him, even if he gets nothing from it, but the pleasure of seeing it.' (Smith, 1759, part I, ch. 1, p. 1, para 1). In this sense, human virtues such as benevolence (doing something for another without expecting anything in return), justice (punishing harmful behaviour inflicted on someone), as well as prudence and propriety (following rules of conduct during interaction with others) set the frame in which mutually beneficial trade is possible. In his excellent essay, *Adam Smith: A Primer*, Butler (2007, pp. 16-17) emphasises that due to human nature, there is no need for constant attention from governments, as a harmonous, peaceful, and efficient social order will develop (unintentionally) from any group of virtuous individuals. The economic ideas presented by Smith in *An Inquiry into the Nature and Causes of the Wealth of Nations* were very popular and found widespread adoption in the government of the United Kingdom (Butler, 2007, p. 22) with Smith's metrics for determining a nation's wealth, the gross domestic product, continuing to be used in the present.

[2] Such as the division of labour, incentives as motivation, money, and private property
[3] The page number refers to the book format as it is published on earlymoderntexts.com

Smith also deliberated upon the mechanisms humans use to determine, which commodities are valuable and which are not. He described an apparent water-diamond paradox, which he could not explain, stating that something essentially useless (a diamond) has a much higher value on the free market, than that which is necessary for survival (water) does (Smith, 1776, Book I, Chapter 4, p. 12)[4]. Seeking to resolve this, he remained at a descriptive level discerning between goods that have a 'value in use' such as water, and goods that have a 'value in exchange' such as diamonds. These kinds of observations could not be resolved in the field of moral philosophy for 100 years until the marginal utility theory was proposed in the 19th century and economics was established as a discipline separate from philosophy (Caplin & Glimcher, 2014).

This brief historic review shows that scholars have long contemplated which decisions are beneficial to society (in economic, moral, and social ways) and how human nature can contribute to these aims accounting for the driving forces behind pain and pleasure. In the next section, the main developments in the field of economics with a focus on risky and intertemporal choice and the theories relevant for this thesis are introduced. A full account, which would also include the psychological perspectives and influences will therefore not be discussed as they do not influence the task models used in the present thesis. It should be noted, however, that economists continue to acknowledge the existence of motivational drivers and their influence on decision-making. They stated that these motivations cannot be measured directly but may be assessed indirectly as utility by observing choice behaviour. The economist Alfred Marshall wrote in this regard: 'Utility is taken to be correlative to Desire or Want. It has been already argued that desires cannot be measured directly, but only indirectly by the outward phenomena to which they give rise (…)' (Marshall, 1920, p. 78).

1.2. Normative decision theory

In the 19th and 20th centuries, decision-making was approached with two questions, namely "how *should* an agent decide in a given situation?" and "how does the agent *actually* decide?" as well as what the underlying reasons/mechanisms for this choice are. Classical economists such as Samuelson (1937) pointed out that the utility for any commodity can only be inferred by observing choice behaviour without being able to make strong claims about the shape of the potential underlying utility function. This implies that there exists not one, but many possible classes of functions. Specifically, he wrote:

[4] The page number refers to the book format as it is published on earlymoderntexts.com

'As has long been known, the behaviour of an individual in the market, when confronted with various price combinations and under limitations of various incomes, is not in itself sufficient to determine the form of his utility function, but can only give us at best a system of indifference loci to which an infinite number of utility indexes might have given rise, integrability conditions being met' (p.155).

Importantly for the normative approach, Samuelson introduced the usage of axioms (premises accepted as truths within a theoretical framework) making it possible to (1) include all classes of utility functions that contain these axioms and (2) define falsifiable hypotheses based on these axioms and test all classes at once (Caplin & Glimcher, 2014). Should individuals, for example, be observed to choose option *a* when option *b* is also available one may assume that they either prefer option *a* over *b* ($a > b$), value option *a* at least as good as option *b* ($a \geq b$) or view options *a* and *b* with indifference ($a \sim b$). What follows is that any utility function that rests on the assumption that individuals prefer *b* over *a* ($b > a$) can be falsified by observing any of three types of behaviour delineated in the previous sentence.

More generally, utility functions are intended to reflect the notion that outcomes with greater utility are preferred to those with less utility and can be ordered accordingly, which is only possible if at least three axioms are met. The first of these is asymmetry, which states that for any pair of outcomes represented by the variables *a* and *b* if $a > b$, then $b > a$ cannot, at the same time, be valid. The second axiom, completeness, was applied above and requires in its general form that exactly one preference is given for *a* and *b*, either $a \geq b$, $b \geq a$, or $a \sim b$ (*a* and *b* are equally preferred). The third axiom is transitivity, which states that if $a > b$ (*a* is preferred to *b*), and $b > c$ (b is preferred to *c*), then $a > c$ (*a* is preferred to *c*). If this axiom is violated and $c > a$ (c is preferred to *a*) then a 'money-pump' scenario is likely to result, in which an observer can offer *b* for *c* at an additional cost, such as a supplementary payment (e.g. 1 Euro), as well as *a* for *b* and a 1 Euro payment and finally, because of their intransitive preference, *c* for *a*, again for a 1 Euro payment. As a result the agent will be back at the point from which they started, but short 3 Euro, which is deemed irrational behaviour.

Using these logically consistent formalisms enabled economists to define falsifiable computational models to predict behaviour and/or to validate their computational model using observed data. These models are limited inasmuch as they are built on rank-ordered choice observations and therefore only retain their meaning for monotonically (increasing) transformations. In the realm of probabilistic outcomes von Neumann and Morgenstern (1953)

introduced further axioms to better define the unobservable shape of the utility function. Building on the previous axioms noted above, they introduced that of continuity, which states that if an agent has three options (*a*, *b*, and *c*) where *a* and *c* are lotteries[5] and *b* is certain, and if the agent's preference are $a > b > c$, then there has to exist a probability (p) that the agent is indifferent to either *b* or one of the lotteries *a* or *c* ($b \sim p*a + (1-p)*c$).

The other axiom is independence, which states that if an agent prefers lottery *a* over lottery *b*, this preference should not change if another lottery (*c*) with an equally likely outcome is added to each lottery ($p*a > p*b \rightarrow [p*a; c] > [p*b; c]$). If an agent follows all these axioms, he is able to assign to each choice option (*a*, *b*, *c* …) a utility according to some function, which is unobservable but allows an observer to draw several conclusion from the agent's behaviour, who behaves as if he: (1) computes the expected utility (EU) of each option (X) by multiplying utility (u(x)) and probability (p(x)), $EU(X) = \sum_x u(x) * p(x)$; (2) ranks the expected utilities of each option; and (3) chooses the option with the highest expected utility and, therefore, maximises expected utility (i.e. demonstrated rational behaviour). Due to its elegant axiomatic foundation, expected utility theory became the prevailing view on economic choice in the presence of risk/uncertainty for nearly half a century.

As outlined above, rational behaviour in situations with uncertain outcomes requires that an agent weigh each option according to its utility and probability. There are, however, also situations in which the utility of an option (i.e. its reward) is not weighted by the likelihood of its occurrence, but rather the moment that it occurs (delay). These kinds of decision dilemmas are called intertemporal choice (or delay discounting) problems. In the realm of economics, John Rae and Eugen von Böhm-Bawerk discussed them as investment and resource allocation problems (for an excellent summary see Frederick, Loewenstein, & O'Donoghue, 2002). Rae states *'The sociological theory of capital'* (1834) that the extent to which humans are able (or willing) to delay gratification depends on the 'affective desire of accumulation' described as 'the determination to sacrifice a certain amount of present good, to obtain another greater amount of good, at some future period (…)' (p. 53). This affective desire should be positively influenced by concepts arising from moral philosophy (as outlined above) such as happiness relating to a future good, or intellectual and moral powers (p. 58). On the other hand, the uncertainty of life, lack of reason, and the pleasure received from immediately gratification, compared to a good only available after some period of time (pp. 53-54) exerts a negative effect.

[5] Lotteries in the economic sense are choice options with uncertain outcomes

In a manner similar to risky/uncertain choice, neoclassical economists sought to find a normative account for intertemporal choices, culminating in the discounted utility (DU) model. In this model, suggested by Samuelson (1937) which was later axiomatically derived and formalized (Fishburn & Rubinstein, 1982; Koopmans, 1960), an agent is assumed to devalue the utility of a certain amount with a constant proportional rate per unit of time in an exponential fashion if he follows the axioms of completeness, transitivity, continuity (described above), as well as two further axioms, monotonicity, and stationarity. Monotonicity states that the preference between two options does not change if they are delayed by the same amount, if $a > b$ then $(a, t) > (b, t)$, where t is time. Stationarity, holds that indifference between two delayed options depends only on the difference between the times, and not on the time of the first outcome (cf. Kable, 2014, p. 175). The DU function can thus be expressed as:

$$DU(a, t) = \delta^t * U(a),$$

where $DU(a, t)$ is the discounted utility of option a at time t, $U(a)$ is the utility of the option received immediately, and δ is the discount factor ($0 < \delta < 1$). Similar to the EU model discussed above, the DU model provides an elegant (albeit parsimonious) means of aiding humans in deciding if they are to maximise the rate of an internal utility function.

1.3. Descriptive decision theory

Due to their simplicity and mathematical foundations, normative models of decision-making were popular as they seemed to offer solutions to decision problems without considering non-observable variables such as psychological states assuming humans to be rational decision-makers (*'homo oeconomicus'*). However, in the second half of the 20th century economists and psychologists devised experiments in which they manipulated outcomes (mainly monetary, but also food in nonhuman animals), a concomitant attribute (waiting time, probability), as well as the manner in which each was framed. Normative models do not possess strong assumptions about how individuals weigh outcomes (i.e. money) whereas, marginal utility theory, for example, does. On the other hand, the models do exert strong restrictions, via their respective axioms, particularly concerning the manner in which information relating to delay and probability is weighted. For example, individuals who prefer a larger over a smaller outcome both available immediately should retain this preference if both outcomes are delayed by the same amount (the monotonicity axiom). Analogously, the preference for the lotteries A1 (a 25% probability of receiving 1000 Euro) and A2 (a 20% probability of receiving 2000 Euro) should

not change if the probability for each lottery is reduced by the same amount: A1 (a 2.5% probability of receiving 1000 Euro) and A2 (a 2% probability of receiving 2000 Euro). This has subsequently been termed the independence axiom.

In contrast to the predictions of the normative models, empirical evidence demonstrated two noteworthy results. First, systematic violations were noted that were replicable in both human and nonhuman animals, and second, some were comparable for intertemporal and risky choice (Prelec & Loewenstein, 1991). In both domains, humans (and other animals) violated the independence (risky choice) and monotonicity/stationary (intertemporal choice) axioms, resulting in *preference reversals*. In the risky domain, this has been famously demonstrated by the Allais paradox (Allais, 1953) that showed participants were risk-averse for moderate to high probabilities, while risk-seeking for small probabilities. With respect to intertemporal choice, Strotz (1955) reasoned that an individual may have several reasons for violating the axioms of the DU model (i.e. changes in tastes, distrust in the future, a lack of education, etc.) and resorts to myopic pleasures either owing to the failure of self-control, or a change in choices made at an earlier time period attributable to a shift in preferences. These theoretical insights and concurrent formal changes in the hypothetical discounting rates have been empirically corroborated. This occurred for the first time in an experiment conducted by Richard Thaler (1981) revolving around hypothetical monetary amounts. Participants were presented with different amounts (small/medium/large) and asked how much (more) they required if they were to be indifferent to receiving it after one/three/six/twelve months or ten years. Thaler showed that the (implicit) discounting rates were much higher for shorter delays (preference for the immediate amount) indicating higher discounting for near delays compared to more distant ones, contradicting exponential discounting.

Almost contemporaneously, direct evidence for preference reversals was provided in a seminal study by Solnick, Kannenberg, Eckerman, and Waller (1980) who used a negative reinforcer (90 dba white-noise) and gave their participants two options to reduce its volume: (1) either immediately (or after 15 seconds) for 90 seconds or, (2) after 60 seconds for 120 seconds (or after 30 seconds for 150 seconds, respectively). One major finding was that participants preferred the immediate (but shorter duration) option, switching to the longer duration option when the choice outcome was delayed by 15 seconds.

Two widely observed phenomena in intertemporal and risky choice behaviour are the *immediacy* and *certainty effects*, respectively. The certainty effect was also first described by

Allais (1953) who noticed that humans show a disproportionally strong preference for certain outcomes, even if the alternative lottery has a higher expected value[6], demonstrating a general tendency to avoid risk if a sure alternative is possible. In a similar fashion and also in line with the experiment by Solnick et al. (1980), in intertemporal choices individuals demonstrated a strong bias for outcomes that are experienced immediately. Both the certainty and immediacy effects can be understood as special cases of a violation of the independence and stationary axioms, respectively.

Differences in how gains/losses are perceived have also been observed in intertemporal/risky choice, with the associated changes in behaviour termed the *sign effect*. In the intertemporal choice domain, delayed gains have been observed to experience steeper discounts compared to losses (Benzion, Rapoport, & Yagil, 1989; Estle, Green, Myerson, & Holt, 2006; Murphy, Vuchinich, & Simpson, 2001; Thaler, 1981), contradicting the DU model, which assumes loss aversion and the attractiveness of gains are similarly discounted. In the risky choice domain, Kahneman and Tversky (1979) introduced the 'reflection effect' for probabilistic prospects to describe their observation that individuals preferred smaller but certain gains over larger but probabilistic ones while preferring larger but probabilistic losses over smaller but certain ones.

In contrast to the sign effect, in which individuals are expected to only process the objective characteristic of an amount (i.e. whether it is positive or negative) the *framing effect* occurs when the same problem is presented in different ways and affects decisions by shifting the implicit point of reference created by the presentation. Tversky and Kahneman (1981) showed this with the Asian disease problem that results in 600 deaths and whose impact can be contained by one of two, mutually-exclusive programmes. In one frame, the first programme saves 200 people while the second programme has the potential to save all 600, albeit with a success rate of 33%, compared to a failure rate of 66% in the event of which, no one is saved. In the other frame, if the first programme is adopted, 400 people will die, while for the second programme, there exists a 33% chance that no one will die, and a 66% chance that all 600 people will die. The majority of the first sample group decided to be risk-averse when presented with the first frame ('save' 200 people) while the second sample group opted for a risk-seeking approach in the second frame (risk 600 deaths to save everyone, as opposed to 400 deaths to save a certain, albeit smaller amount). Such framing effects have also been observed in

[6] In Allais' example, individuals preferred a certain amount (100 million French Francs) over a lottery that offered them a 10% chance of receiving 500 million French Francs, an 89% chance of receiving 100 million French Francs and a 1% chance of receiving nothing.

intertemporal choice (Loewenstein & Thaler, 1989; Loewenstein, 1988) and have been termed 'delay-speedup-asymmetry'. For example, in the delay frame, participants were asked how much compensation they would want for waiting 6 months to use a $100 gift card otherwise available today. In the sped-up frame, other participants were asked how much they would pay to receive a $100 gift card today, as opposed to six months in the future. In the first frame, participants demanded an average compensation of $23.85, while in the second frame, they were willing to pay an average of $10.17 (Loewenstein, 1988). Hence, immediate consumption options that are delayed feel more punishing than delayed consumption that can be made available immediately.

Finally, the *magnitude effect* has been observed in both choice domains and demonstrates that larger gains are less discounted for a given delay than smaller gains are for an intertemporal choice (i.e. Benzion et al., 1989; Chapman & Weber, 2006; Chapman & Winquist, 1998; Thaler, 1981) and that individuals are risk-seeking for smaller gains albeit risk-averse for larger ones (Chapman & Weber, 2006; Cruz Rambaud & Sánchez Pérez, 2018; Weber & Chapman, 2005).

1.4. Intertemporal and risky choice: towards a common discounting framework

These and other anomalies (described elsewhere, i.e. Frederick et al., 2002; Kahneman & Tversky, 1979; Tversky & Kahneman, 1992) cannot be explained with the standard normative EU/DU models and have led to the conclusion that humans and other animals do not behave as if they maximise an internal reward rate function. Rather, they weigh the attributes of an option, for example its utility (i.e. monetary gain/loss) and availability (i.e. probability or delay) in an apparently irrational manner. Certain/immediately available gains are preferred over delayed/uncertain ones while the opposite is observed for losses (reflected in lower temporal discounting rates and risk-seeking behaviour to avoid sure losses).

To find a function with a shape capable of accounting for the anomalies in the intertemporal choice domain, several experiments with nonhuman animals were conducted in which reinforcement schedules provided access to either a smaller/sooner or larger/later food reward. Several functions (i.e. exponential, square root, hyperbole) have been suggested in line with the observed behaviour (cf. Ainslie, 1975; Ainslie & Herrnstein, 1981; Rachlin & Green, 1972). Based on this previous research Mazur (1987) proposed the now well-known and widely used hyperbole discounting function to explain animal behaviour, formalised as:

$$SV = U(x) / (1 + kD),$$

where SV is the subjective value (or discounted utility) of the delayed amount x, U(x) is the utility of the amount if it was immediately available, D is the delay of x, and *k* is the discount rate. This function allows for inconsistencies observed in experiments such as preference reversals, and is therefore steeper for shorter time intervals and flatter for longer ones.

In contrast to the development of the discounting framework for intertemporal choices, prospect theory (Kahneman & Tversky, 1979; Tversky & Kahneman, 1992) is arguably the most apt model in the risky choice domain for explaining the anomalies. Similar to the EU model, it assumes that the utility of a lottery is a combination of its amount and probability. The main differences are, however, that individuals transform amount to a value, while ascribing probabilities with a certain weight. Hence, for a certain outcome (gain/loss) prospect theory assumes diminishing marginal utility (i.e. the gain/loss of 2000 Euro does not feel twice as good/bad as the gain/loss of 1000 Euro). Furthermore, a prominent difference with the EU model is the fact that prospect theory does not assume that outcomes are processed in relation to total wealth but rather separately to a reference point. This reference point categorises outcomes as gain or loss, depending on the individual status quo. For example, a raise in salary of 500 Euro may feel positive given the status quo. On the other hand, if other colleagues receive a raise of 1000 Euro, the reference point shifts accordingly and the 500 Euro net gain will be perceived as a loss. To explain why individuals are risk-averse for gains and risk-seeking for losses, the s-shape curve of the value function is concave for gains and convex for losses. The shape signifies that a gain of 100 Euro does not elicit as many positive feelings as the negative feelings arising from a loss of 100 Euro. The value function also explains the phenomenon of *loss aversion* in the context of mixed prospects. Tversky and Kahneman (1992) suggested that if a prospect contains both a gain and a loss, the loss looms larger than the gain. Accordingly, a gain has to be around twice as large as a loss in order to be attractive. The probability weighting function accounts for the observations that small probabilities are overestimated while moderate-to-high probabilities are underestimated. For example, probability changes of 0.05 from the endpoints of the function w(p) = 0 or w(p) = 1 (where w(p) is the weighted probability) have a much stronger weighting effect[7] that do changes in the middle of the function, e.g. a change from 0.6 to 0.65.

[7] A change from p = 0 to p = 0.05 leads to an overestimation in the probability weighting because an outcome shifts from impossible to possible (the possibility effect) while a decrease from p = 1 to p = 0.95 leads to an underestimation of the probability because an outcome shifted from certain (certainty effect) to likely

Although prospect theory has substantially advanced our understanding of how value and probabilities are internally represented, the main interest was to identify cognitive mechanisms that drive decision-making (such as the effect of framing, flipping the sign, etc…) rather than the choices themselves. Therefore, participants usually made only one or two choices but never longer sequences, depending on the choice dilemma (i.e. the Allais paradox or the Asian Disease problem). Another way to look at probabilistic choice is through the lens of behavioural experimental paradigms based on nonhuman animal research. In their intriguing article, Rachlin, Logue, Gibbon, and Frankel (1986) argue that risky choice can be understood as analogue to intertemporal choice problems where the concept of choosing the gamble with the highest expected value is replaced with the concept of maximising an overall reinforcement rate according to previous choices. They present an example of a lottery (realised as a wheel of fortune) where you can win *x* dollars with a 33.3% (or 1/3) probability and a 66.7% (or 2/3) probability of winning nothing. The expected value of the lottery therefore is x/3. If this lottery is chosen multiple times (i.e. rotating the spinner), the rotation time is c seconds and the experimenter waits on average t seconds between each spin (inter-trial interval, ITI), then the expected delay the participant has to wait until the first win can be described with the following waiting time function:

$$D = (t + c / p) - t \text{ seconds},$$

where D is the expected delay to the first win and *t* is subtracted if there is no ITI before the first spin. In this case, $D = (t + c / 1/3) - t = 3*(t + c) - t$. The expected average reward rate would be $x/D = x/3*(t + c)$ dollars per second. A choice between a smaller certain and a larger probabilistic gain can consequently be perceived as the choice between an immediate and a delayed reinforcer. Furthermore, Rachlin, Raineri, & Cross (1991) showed that if *c* is so small relative to *t* (when the spinning time is very short compared to the ITI) that it can be neglected, the equation for D reduces to $D = (t/p) - t$, which can be rearranged as $D = t((1/p) - 1) = t\Theta$, where $\Theta = (1/p) – 1$, which denotes the 'odds against'. Substituting D from the hyperbolic discounting function with Θ yields the hyperbolic probability discount function:

$$SV = U(x) / (1 + k\Theta),$$

where SV is the subjective value (or discounted utility) of the probabilistic amount x, U(x) is the utility of the amount if it is surely available, and *k* is the discount factor. Studies using the discounting framework provided empirical evidence that the function preserves several properties that have been described by prospect theory, such as preference reversals (Allais'

paradox) and the sign effect (gains are more steeply discounted than losses, indicating risk-aversion for gains and risk-seeking for losses; Estle et al., 2006; Rachlin et al., 1991). As both the risky and intertemporal choice phenomena can be well described using a hyperbolic discounting function, the question arose as to whether there exists a common process accounting for both decision-making strategies. A thorough review conducted by Green and Myerson (2004) came to the conclusion that this hypothesis is unlikely because amount has opposing effects on discounting behaviour whereas increasing gains decrease discounting for intertemporal choices, while increasing discounting for risky choices. This finding has been replicated in experiments by Estle et al. (2006), in which no significant effect of smaller and larger losses on intertemporal/probability discounting was found.

Additional evidence for separate valuation systems is presented by Peters and Büchel (2009) who used functional magnetic resonance imaging (fMRI) to investigate brain signals during intertemporal and probabilistic choices. After subtracting the probability discounting contrast from the intertemporal choice contrast, they found an activation cluster in the medial frontal pole, a region associated both with future thinking and the representation of future outcomes. On the other hand, valuation signals unique for probability discounting encompassed regions in the parietal and occipital regions, which the authors cautiously interpreted as abstract number coding, such as expected value calculations, which are not possible in intertemporal choices.

Despite the fact that intertemporal and probabilistic choices may have separate underlying psychological processes which cannot be resolved with simple economic models, the hyperbolic discounting framework still maintains strong predictive power. This has been demonstrated in a recent report in which hyperbolic discounting in the intertemporal choice domain has been compared to more complex heuristic models based on psychological principles (for example the ITCH model prepared by Ericson, White, Laibson, & Cohen (2015)) that use up to five parameters (Wulff & van den Bos, 2018). Here, depending on the model evaluation method, the performance of the single-parameter hyperbolic model is comparable to the multi-parameter heuristic models. The conspicuous advantage is that far fewer trials are needed to estimate one parameter compared to five parameters, making the model very efficient, and allowing for nonhuman animal choice behaviour to be very well described with hyperbolic discounting (Vanderveldt, Oliveira, & Green, 2016). In the probabilistic choice domain, the discounting framework is more attractive for similar reasons, as it requires only one free parameter to be estimated compared to prospect theory, which uses three parameters for the

power function and two parameters for the probability weighting function (Tversky & Kahneman, 1992).

In contrast to probability discounting paradigms that measure their constructs (risk-aversion and risk-seeking, respectively) using only gain or only loss options, loss aversion is usually measured using mixed prospects that demonstrate both the chance to win or lose an amount (Tversky & Kahneman, 1991; Tversky & Kahneman, 1992). As previously described in the paragraph on prospect theory above, loss aversion is the observation that the loss of an amount generates more negative feelings than receipt of the same amount does positive feelings. As previous studies have shown (Frydman, Camerer, Bossaerts, & Rangel, 2011; Tom, Fox, Trepel, & Poldrack, 2007), instead of estimating all parameters from the prospect theory functions, a more practical way to accomplish a similar result is to assume linear value processing and negligible distortions for a probability of p =.5, which reduces the formula for computing loss aversion λ to a simple linear function

$$SV = 0.5*(G – \lambda L),$$

where SV is again the subjective value of the mixed gamble, G the prospective gain, L the prospective loss, and λ the loss aversion parameter. Such choice dilemmas that require agents to decide between amounts with a certain value and delay/probability are commonly described as value-based decision-making (VBDM) tasks.

This overview has shown that humans and other animals do not behave rationally with regards to maximising an internal utility rate, which can be normatively described, but rather show several decision-making biases that can be addressed with descriptive choice models. Motivated by these findings, extensive research has been conducted from an evolutionary perspective (i.e. Santos & Rosati, 2015) to quantify the stability of discounting behaviour with respect to heritability and trait features, in particular, intertemporal choice (for example Anokhin, Golosheykin, Grant, & Heath, 2011; Anokhin, Grant, Mulligan, & Heath, 2015; Odum, 2011). Another important field, computational psychiatry, uses VBDM parameters as behavioural markers (especially intertemporal choice) to investigate their relationship to mental disorders such as addiction/substance abuse (Amlung, Vedelago, Acker, Balodis, & MacKillop, 2017; Bickel et al., 2019), eating disorders (McClelland et al., 2016) and personality/affective disorders (cf. Story, Moutoussis, & Dolan, 2015) providing evidence that steeper temporal discounting is positively associated with symptom severity (except for anorexia nervosa, where the opposite pattern has been observed). Due to their important role in the public health sector

(Lopez-Guzman, Konova, & Glimcher, 2018) VBDM parameters have become an integral aspect of the newly-founded Research Domain Criteria (RDoC) with a recent report highlighting the importance of intertemporal choice (Lempert, Steinglass, Pinto, Kable, & Simpson, 2018).

While the VBDM parameters may serve as an endophenotype for mental disorders, a great deal of interest in the biological foundations of VBDM, specifically in the neural (structural and functional) correlates of the brain and the role of neurotransmitters has also been generated. Its researchers, who primarily hail from the fields of economics, neurosciences and cognitive psychology began to collaborate in the late nineties (as functional magnetic resonance imaging, fMRI became increasingly available) on questions such as whether the brain processes choices according to economic theories (i.e. value maximisation), whether biophysical constraints may explain deviations from normative models and, if so, what evolutionarily meaningful parameter spaces they constitute. These researchers founded the Society for Neuroeconomics in 2005 (cf. Glimcher & Fehr, 2014; McCabe, 2008; Reuter & Montag, 2016). This young interdisciplinary field established fMRI as a key tool in determining the neural correlates of decision-making behaviour in humans as well as pharmacological interventions to target specific neurotransmitter systems. The most important findings for this thesis with respect to brain networks and intertemporal choice are presented in the following section.

1.5. Brain networks associated with intertemporal choice

Although the primary focus of this work, with respect to neural correlates, is on ITeCh, there also exists a large body of evidence suggesting that the main features of probabilistic offers (gain/loss amount, probability, subjective value) are processed in brain regions that are also implicated in ITeCh such as the (ventral) striatum (VS), the medial prefrontal cortex (MPFC), posterior cingulate cortex (PCC), orbitofrontal cortex (OFC) where activity in these regions is positively related to increased values of all features (for reviews see Cardinal, 2006; Haber & Knutson, 2010; Tobler & Weber, 2014; Trepel, Fox, & Poldrack, 2005).

Research in neural correlates of ITeCh has identified several brain regions involved in the decision-making process. Some of these comprise the well-known ‘reward circuit’ (Haber & Knutson, 2010) that consists of corticolimbic loops, known to be connected to the processing of a large variety of rewards. A recent meta-analysis, based on human fMRI studies, suggests that a core valuation network includes the ventromedial prefrontal cortex (VMPFC/OFC and

VS) and valuates the magnitude of a reward in addition to its attributes such as risk, uncertainty, valence, and modality (Clithero & Rangel, 2014). Further brain regions, which comprise the extended valuation network are the amygdala, thalamus, substantia nigra (SN), ventral tegmental area (VTA), and hippocampus (Haber & Knutson, 2010; Peters & Büchel, 2011). Other regions that are repeatedly found in ITeCh are the lateral cortical ones such as the dorsolateral prefrontal cortex (DLPFC), inferior frontal gyrus (IFG), posterior parietal cortex (PPC), the temporoparietal junction (TPJ), and the anterior cingulate cortex (ACC) (Ballard & Knutson, 2009; Kable, 2014; McClure, Laibson, Loewenstein, & Cohen, 2004).

The exact role and interplay of the involved regions has yet to be satisfactorily established, however, two competing theories and a relatively new approach have emerged that seek to explain the findings. The first of these is the 'dual-systems' theory, which essentially states that intertemporal choices are guided by two competing systems: the β or 'impulsive' system and the δ or 'deliberate' system. This idea is reminiscent of the hot/cool systems introduced by Metcalfe and Mischel (1999). The hot system (β) denotes a myopic, emotional, and irrational decision-making style while the cold (δ) system is epitomised by a reflective, rational, and self-controlled decision-making style. In their seminal fMRI study, McClure et al. (2004) found neural correlates of these systems: a corticostriatal network consisting of the VS, MPFC, and PCC that was active when an immediate amount option was available (β) but not when both amounts were associated with a delay and a cortical network comprising the DLPFC, ventrolateral prefrontal cortex (VLPFC) and the lateral orbitofrontal cortex (LOFC) that was active when participants chose the delayed amount (δ). They interpret their findings as showing that the β regions lend additional weight to the subjective value of the immediate option, competing with the δ regions. Interestingly, Cardinal, Winstanley, Robbins, and Everitt (2004) note with respect to animal research on rats that 'AcbC-lesioned (nucleus accumbens core) subjects were rendered impulsive in their choices: they exhibited a profound deficit in their ability to choose a delayed reward and persisted in choosing impulsively even though they were made to experience the larger, delayed alternative at regular intervals' (p. 39). This finding has been frequently observed and indicates that the VS promotes choices for the delayed amount.

Criticism regarding the dual-systems account was raised by Kable and Glimcher (2007) who stated that McClure et al. (2004) did not parametrically modulate brain activation with subjective value (only controlled for it in their GLM) and used a dummy variable coding approach instead to model decision epochs. Kable and Glimcher (2007) further argued that the

corticostriatal regions may not signal immediacy per se but rather track the subjective value of all available amounts with sooner/immediate amounts are perceived as inherently more valuable. To test their hypothesis, they devised an experiment in which the immediate amount was fixed and only the delayed amount varied over trials. Indeed, they found significant activations in the β regions (VS, VMPFC, and PCC) reported by McClure et al. scaling with the subjective value (rather than with amount magnitude or delay) of the delayed amounts at all delays, which is at odds with the assumption that the β regions exclusively value immediate amounts. Their finding suggests that there exists only a single-system consisting of corticostriatal brain regions that evaluate available options according to a hyperbolic discount function resulting in a choice for the option with the higher subjective value.

A third approach has been introduced by Figner et al. (2010) who propose that the choice preference for an intertemporal choice is not merely driven by 'pure' valuation alone as suggested by the dual- and single-system accounts, but also involves a self-control mechanism that does not influence the valuation process *per se* but may override its outcome, which in turn leads to a change in choice behaviour and may in part explain preference reversals. This account is motivated by their repetitive transcranial magnetic stimulation (rTMS) experiment in which they transiently disrupted the left or right lateral prefrontal cortex (LPFC) or delivered a sham stimulation to their participants engaged in an intertemporal choice and a valuation task. Their primary finding was that the participants in the left LPFC group more often chose the immediate amount when it was available (compared to sooner/later trials) than the two other groups, but did not differ in the subsequent valuation task on evaluating the attractiveness of low, medium and high amounts available immediately. They concluded that the results support their self-control explanation, because offer valuation did not differ between groups, while the left LPFC group showed a stronger tendency to choose the smaller immediate amount over the larger later amount, but not the smaller sooner over the larger later amount, demonstrating impulsive preference reversals in the face of immediately available amounts.

Although there is agreement on the brain regions generally involved in intertemporal choice, the specific contribution of each brain region remains heavily debated, especially in the context of the single- and dual-systems account (Koffarnus, Jarmolowicz, Mueller, & Bickel, 2013; Monterosso & Luo, 2010). The main commonality linking these three views is that there is an existing valuation system consisting of cortical and subcortical brain regions. However, while the single- and dual-systems account assume that choice results directly from valuation, where the option with the highest subjective value is consequently selected, the self-control approach

assumes that an interfering system can override the valuation outcome and bias choice behaviour, for example, towards the delayed option yielding a larger reward. This is underscored in an interesting review (Scheres, de Water, & Mies, 2013) that contrasts all three approaches in light of the existing (fMRI) evidence and states: 'In sum, data from fMRI studies so far do not provide conclusive evidence in favour of one specific account. Rather, data support all accounts to some extent, and we suggest that integration of the accounts may be feasible. Clearly, future research is needed to refine theoretical accounts of the neural basis of TD (temporal discounting)' (p. 532). The main reason for this ambiguity is that the task designs do not allow direct testing of the views against one another, while the self-control theory is unspecific about the brain regions involved in the valuation process. Accordingly, future studies are needed to address these issues. Nevertheless, it is tempting to assume that a valuation system exists in the brain that preferentially processes amount related information and a cognitive control network that is mainly involved in the processing of delay information (Ballard & Knutson, 2009; Peters & Büchel, 2011).

Intertemporal and risky choice processes are known to be influenced by neuromodulators such as serotonin that manipulate neuronal activity and have been suspected to represent states and expectations which guide decision-making. In the following sections, an outline of the relevant aspects of serotonin and how it has been implemented in computational approaches to explain decision-making leading to the main research questions of this thesis are presented.

1.6. Serotonin

Serotonin (5-Hydroxytryptamine, 5-HT) is a phylogenetically ancient neurotransmitter that is synthesized from the essential amino acid tryptophan. 5-HT was first identified in enterochromaffin cells (and accordingly named 'enteramine') by Erspamer & Viali (1937). In 1952, it was renamed serotonin by Erspamer & Asero (Hornung, 2010). In 1964, Dahlström and Fuxe used histofluorescence techniques to identify that serotonin expressing neurons originated from the raphe nuclei in the tegmentum, an area located within the brainstem. The authors grouped the cell bodies into 9 distinct clusters (B1-B9) along a caudal-rostral gradient with the caudal group (B1-B4) including the raphe pallidus nucleus (B1, B4), the raphe obscurus nucleus (B2), and the raphe magnus nucleus (B3) that all project mainly to the caudal brainstem and the spinal cord. The rostral group includes the median raphe (MR) nucleus (B5, B8), pontine raphe nucleus (B5), the dorsal raphe (DR) nucleus (B6, B7), the caudal linear nucleus (B8), and the B9 nucleus (cf. Hornung, 2010; Okaty, Commons, & Dymecki, 2019).

The main targets of the rostral group (primarily starting from the MR and DR) are the forebrain and the brainstem. More specifically, the rostral group's efferent projections to the forebrain are divided into two parallel routes: the dorsal and ventral pathways (Hornung, 2012). The dorsal pathway projects at the ventral side of the medial longitudinal fasciculus (an association fibre bundle that connects the posterior and anterior parts of the cerebrum), passes through the internal capsule (a larger fibre bundle passing through the basal ganglia separating the caudate nucleus and thalamus from the putamen and globus pallidus) and heads for the lateral cerebral cortex. The ventral pathway starts at the trochlear nucleus and passes lateral to the MR, reaching the ventral tegmental area near the dorsal limits of the interpeduncular nucleus within the midbrain to extend into the medial forebrain bundle reaching the basal forebrain and the medial cortex. Fibres of the ventral and dorsal pathways merge transiently at the midbrain-forebrain junction. The main target sites of the rostral DR are the cerebral cortex, neostriatum, amygdala, thalamus, lateral septum, and substantia nigra, while the caudal DR projects to the hippocampus, entorhinal cortex, and locus coeruleus. The MR sends projections from its midline primarily to the basal forebrain, the septal region, and hippocampus while the dorsal and lateral parts project to the amygdala, substantia nigra, thalamus, and mammilar nuclei (Hornung, 2012). As a result, the 5-HT pathways encompass all important brain regions that are associated with reward and decision-making (i.e. striatum, nucleus accumbens, amygdala, and frontal cortex).

1.7. Serotonin at the synaptic level

It is well known that 5-HT is synthesized in the brain from the essential amino acid tryptophan (i.e. Hasegawa & Nakamura, 2010) and is supplied by an individual's dietary intake. It is estimated that 3% of tryptophan goes towards 5-HT synthesis throughout the body, with only 1% used for 5-HT synthesis in the brain, while around 90% of tryptophan is synthesized to kynurenine (Richard et al., 2009). In the blood stream, tryptophan is around 95% albumin-bound and 5% free, with only this latter percentage passing through the blood brain barrier via the large neutral amino acid transporter (LAT1). In the brain's extracellular fluid, tryptophan is transported into a serotonergic neuron via the high-affinity tryptophan receptor after which it is hydroxylated to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase 2, which is only half saturated at normal tryptophan concentrations, hence providing the potential to increase 5-hydroxytryptophan synthesis up to two-fold supposing that the concentration of tryptophan increases. Following this, 5-hydroxytryptophan is rapidly converted to 5-HT by aromatic L-amino acid decarboxylase enzyme to either be metabolized by monoamine oxidase B to 5-

hydroxyindole acetaldehyde and further converted to 5-hydroxyindoleacetic acid by aldehyde dehydrogenase or stored in synaptic vesicles and released into the extracellular fluid. (Ruddick et al., 2006; Zepf, 2012). Upon release following stimulation, 5-HT can activate pre- and postsynaptic 5-HT receptors that show a large variety of subtypes and functions (Barnes & Sharp, 1999). Based on cloning techniques (Sealfon, 1995), the 5-HT receptors can be grouped into seven families (5-HT1-7) comprising at least fourteen structurally and functionally distinguishable sub-receptors (Charnay & Léger, 2010; Hoyer et al., 1994). The primary removal mechanism for extracellular 5-HT is provided by the serotonin transporter, which returns 5-HT to the presynaptic cell to either be recycled into vesicles or metabolized. In the peripheral nervous system, 5-HT can also be synthesized by gut neurons or enterochromaffin cells to serve several functions, for example as a hormone, but it cannot pass through the blood-brain barrier (Bouchaud, 1972). Therefore, the central and peripheral 5-HT systems work independently of one another.

1.8. Serotonin in brain development

5-HT has also been suspected to play an important role during brain development as a neurotrophic factor impacting the structural organization and function of serotonergic and non-serotonergic neurons as well as behavioural phenotypes, generating a large body of literature (reviewed in Daubert & Condron, 2010; Deneris & Gaspar, 2018; Gaspar, Cases, & Maroteaux, 2003; Kepser & Homberg, 2015; Kraus, Castren, Kasper, & Lanzenberger, 2017; Lesch & Waider, 2012; Dennis. L. Murphy et al., 2008; Persico, Kalueff, & LaPorte, 2010). One of the reasons why 5-HT is focused upon is that serotonergic neurons are among the first to be developed; in humans they are detectable in the first trimester, start rapidly outgrowing and differentiate during the second trimester by developing cytoarchitectonic organization, refining their brain circuits during the third trimester (Kepser & Homberg, 2015). It has also been demonstrated in mammals and Drosophila that the serotonin transporter (SERT) is already expressed before 5-HT neurite outgrowth and synapse formation, which highlights the importance of tightly regulated 5-HT signalling (Daubert & Condron, 2010). Non-serotonergic neurons, as observed for example in the thalamus, limbic cortex, and hypothalamus of mice brains, are able to transiently uptake 5-HT via SERT, store and release it, all without being able to synthesize it themselves (Gaspar et al., 2003), providing another important mechanism to maintain optimal tonic levels of 5-HT. Numerous animal studies (reviewed for example in Daubert & Condron, 2010; Gaspar et al., 2003) have demonstrated that excessive 5-HT levels

in rodents result in disrupted formation of barrels in the somatosensory cortex, leading to the clustering and segregation of thalamocortical fibres in the visual system. Depleted 5-HT levels on the other hand have detrimental effects on neuronal development disrupting maturation of pyramidal neurons by reducing dendritic arborisation and altering the migration of interneurons, possibly due to the reduced expression of cell-adhesion molecules. These molecules themselves are important for building and maintaining synaptic structures as well as synaptic plasticity (Lesch & Waider, 2012).

The molecular mechanisms through which 5-HT modulates neurite growth, neurogenesis and differentiation/proliferation are extensive and include, among others, intracellular signal transduction pathways, transcriptional factors, the modulation of activity of other neurotransmitters (i.e. gamma - aminobutyric acid, glutamate, norepinephrine, and dopamine) but also – most importantly – the manifold receptor subtypes. Nowadays, the influence of only a few receptor subtypes (5-HT1A, 5-HT1B, 5-HT2A, 5-HT2C, 5-HT3A, 5-HT4, 5-HT6, and 5-HT7), as well as the SERT and MAO-A could be related to neuroplasticity by interacting with many proteins such as brain-derived neurotrophic factor, and N-methyl-D-aspartate extracellular signal-regulated kinases (Gaspar et al., 2003; Kraus et al., 2017; Murphy et al., 2008). As summarized by Kepser and Homberg (2015), these molecular processes do not occur concurrently but during different stages of development (ranging from prenatal to late postnatal) resulting in a large variety of behavioural phenotypes, mainly related to impaired motor activity, sexual behaviour, aggressive behaviour, and disorder-like behaviour (such as depression, anxiety, and autism).

Another line of evidence further demonstrates that changes in structural morphology related to the dysfunction or complete absence of SERT can be observed in corticostriatal pathways that are implicated in reward processing and decision-making. More specifically, this has been shown in SERT knock-out mice, a special strain that are genetically engineered to lack the SERT protein resulting in excessive extracellular 5-HT levels and related morphological changes (Gaspar et al., 2003; Murphy et al., 2008). To examine this, Bearer, Zhang, Janvelyan, Boulat, and Jacobs (2009) injected SERT knock-out and normal mice with ionized manganese (Mn^{2+}), which has paramagnetic properties and used *in vivo* MRI demonstrating that in knock-out mice Mn^{2+} travelled faster from the PFC (injection site) to the basal ganglia (caudate, putamen, and globus pallidus) and accumulated in the SN, VTA and DR, whereas in normal mice Mn^{2+} stopped at the mid thalamus. According to the authors, these findings suggest that SERT knock-out mice have increased neuronal activity in the nigrostriatal and mesocortical

pathways and less in the mesolimbic pathway compared to normal mice. Additionally, they also most likely have more neurons projecting along these two pathways and increased dendritic uptake by neurons in those areas.

Overall, it is evident that 5-HT plays a pivotal role in shaping the connectome in the brain via a complex interplay between serotonergic signalling levels, different receptor subtypes, and the modulation of other neurotransmitters. Accordingly, it is reasonable to investigate how these molecular and systemic phenotypes translate to behaviour.

1.9. A computational approach for serotonin and value-based decision-making

One seminal review that summarized human and nonhuman animal studies suggested an important role for 5-HT in punishment-related inhibition and impulse control in the contexts of novelty, non-reward situations, and learned helplessness in nonhuman animals and a role of low 5-HT levels in human aggression, impulsive conduct, and violent suicide (Soubrié, 1986). Around the same time (1983), one of Deakin's early theories suggested that brain 5-HT modulates the behavioural response to aversive cues or events (cited in Deakin, 2013; Deakin & Graeff, 1991). The authors established that 5-HT induces avoidance behaviour and inhibition of a current action via projections from the DR to a network comprising the amygdala, hippocampus, habenula, and conversely from prefrontal regions to the DR. At the same time, 5-HT also inhibits fight/flight mechanisms that are controlled by the periaqueductal grey (PAG). Perception of an event that may result in imminent death results in suppression of 5-HT release, which activates the PAG and fight/flight behaviour. Another important finding the the authors uncovered was that 5-HT and dopamine project into similar brain regions, especially in the subcortical and cortical regions implicated in decision-making. These interactions between 5-HT and dopamine have also been validated on a functional level by numerous *in vivo* microdialysis and electrophysiological evidence (cf. Di Giovanni, Di Matteo, Pierucci, & Esposito, 2008; Di Matteo, Di Giovanni, Pierucci, & Esposito, 2008).

Although interactions between 5-HT and dopamine have been well known for several decades (i.e. Samanin & Garattini, 1975; Trent & Tepper, 1991) and despite the initial step by Deakin & Graeff, it has been a challenge to establish a model that formally describes and integrates the experimental findings of 5-HT (and dopamine) on the behavioural level. Such an implementation was first conducted by applying the knowledge surrounding 5-HT and dopamine to the reinforcement paradigm.

Reinforcement learning is a computational framework in which an agent (i.e. animal) is placed in an environment consisting of multiple rooms (or 'states') that either contain rewarding or punishing outcomes (Sutton, 1988). The task of the agent is to take actions (by entering rooms) and find an optimal policy to maximize the reward rate over time, all the while minimising punishing outcomes given the current one in any given sequence of adjacent rooms. An important signal to guide learning and decision-making, which rooms to enter and which rooms to avoid, is the temporal difference prediction error. This prediction error incorporates all experienced outcomes and is greater than zero if an outcome is better than expected and smaller if it is worse than expected. Over time when several rooms have been explored and their outcomes experienced, a value function is learned that predicts cumulative future rewards given the current room and the policy relating to which sequence the rooms should be entered in. If the agent, based on the current value function predicting future outcomes, executes its policy for entering rooms, the actual rewards received at the end of the sequence are then compared with the predicted ones given the value function. Any deviation in the amount of expected rewards (either more or less) is signalled by the temporal difference prediction error, which, in turn, is used to update the current value function. As outlined above, the value function represents the overall expected outcome given a sequence of actions that include both positive and negative outcomes if the agent follows the policy. There is, however, another important feature of the value function. Sutton proposes a weighting factor for each room, or state, for each timestep in a sequence of choices that affects the overall expected outcome of the value function. He named this factor the *discount-rate parameter* and argues that it reflects the agent's propensity to value future outcomes on longer timescales. For example, if there is a sequence that entails several small negative outcomes occurring sooner, but a large positive outcome at the end of the sequence, the discount-rate parameter determines the attractiveness of this choice sequence. If the parameter is low (close to zero) the larger, more temporal distant reward is weighed down to such an extent that the value function is negative and it becomes infeasible for the agent to follow the policy. On the other hand, if the parameter is high (close to one), the temporal distance does not affect the larger reward to a great extent, and the value function will be positive.

As the temporal difference prediction error is an important signal for reward prediction and action selection, Doya (2002, 2008) associated this signal with dopamine, while 5-HT may control the discount-rate parameter. This consideration rests on evidence reviewed by Doya (2002) that dopaminergic neurons in the SN and VTA responded to unexpected rewards and

reward-predicting cues, reinforces behaviour with positive outcomes, and is involved in addiction. With respect to 5-HT, Doya cites several studies implicating low serotonin levels in impulsive behaviour and hence in the processing of future consequences. 5-HT possibly interacts with dopamine in the basal ganglia and modulates dopaminergic activity in SN, VTA, and striatum in a way that in event of a positive expected outcome, 5-HT increases dopamine activity, while negative expected outcomes result in an inhibitory effect of 5-HT on dopamine.

An alternative approach to how dopamine and 5-HT are involved in reinforcement learning was provided by Daw, Kakade, and Dayan (2002). They argued similarly to Doya that the prediction error is also governed by dopamine and that 5-HT interacts with it in order to optimize behaviour, nevertheless their approach differs in several obvious ways. First and foremost, the authors assume that 5-HT stands in strict opposition to dopamine in its functioning rather than also being able to reinforce dopamine activity as demonstrated by Doya (2002). To this end, Daw et al. (2002) were among the first to state that both neuromodulators have two separate activation levels: a phasic 'bursting' tone that is characterized by discrete, fast-spiking activity in direct response to a stimulus (i.e. a reward or punishment) that quickly diminishes once the stimuli has passed. The other level has a tonic, rather slow, continuously changing pattern that is hypothesized to increase in the presence of a great deal of phasic activity, decreasing slowly back to the baseline once the phasic activity diminishes. Here, the authors, in line with Doya (2002), postulate that the temporal difference reward prediction error is reflected by phasic dopaminergic activity to carry immediate reward information, but augment the prediction error by subtracting a long-term average reward prediction term from the dopamine component, resulting in the final prediction error. This long-term average reward prediction is meant to be carried by tonic 5-HT levels and increases when rewards occur frequently over time thereby reducing the impact of every new reward and hence the prediction error, while it decreases to baseline when phasic dopamine activity ceases in the face of a lack of rewards. Daw et al. (2002) consider another scenario where an environment exists in which punishments are likely and speculate that, due to previous research on the involvement of 5-HT in aversive processing, a mirrored opponency exists with respect to dopamine and 5-HT activity. In this scenario, phasic 5-HT activity occurs in response to aversive stimuli, while tonic dopamine levels reflect the long-term average punishment prediction. This reasoning is derived from microdialysis studies that show increased dopamine levels in response to aversive stimuli such as foot shocks. Consequently, the authors propose their full temporal difference prediction error to be a

function of phasic activity related to stimuli information (either reward or punishment) and two long-term average predictions about reward and punishment, respectively.

This approach by Daw et al. (2002) was an intriguing step towards a common framework for 5-HT and dopamine as it suggested dedicated roles for the neuromodulators in reward and punishment learning. There were, however, some caveats in their computational theory when applying it to experiments in which the valence of a stimuli (reward – punishment) and the nature of the action (invigorate – inhibit) are orthogonalized, challenging the classic notion that rewards are obtained by invigorating (associated with dopamine) while punishments are avoided (associated with 5-HT) by inhibition. The same research group (Boureau & Dayan, 2011) consequently refined their theory, mainly to allow not only opposing, but also complementary and independent functions of the neuromodulators and the way they influence different kinds of choice behaviour, namely Pavlovian, model-free, and model-based decision-making. They also modified their assumption about the nature of tonic dopamine and 5-HT activity as a result of experimental findings and conclusions presented by Niv, Daw, Joel, and Dayan (2007) in which tonic dopamine levels do not report the long-term average punishment prediction, but rather, the average reward prediction, reversing Daw et al.'s conclusions (2002). One issue that remains unresolved in their theory is the unknown effect of 5-HT on time perception during ITeCh, as highlighted by Doya (2002, 2008). This gap and the role of dopamine and 5-HT on another form of VBDM that was not directly addressed by Boureau and Dayan (2011) was unified in a computational framework by Cools, Nakamura, and Daw (2011) described in the next paragraph.

Similar to the approach by Boureau and Dayan (2011), Cools et al. (2011) were interested in determining how dopamine and 5-HT influence valence and the action of stimuli by phasic and tonic activity and how such a framework could be applied to different kinds of VBDM. At the heart of their framework is the concept of the opportunity costs of time (Niv et al., 2007). The opportunity costs of time essentially determine how vigorously actions should be performed in environments where the occurrence of rewards and punishments varies with time. When rewards are plenty during a given time period, behavioural vigour should be increased to maximise the reward rate, while sloth is costly as more rewards are foregone – the opportunity costs are high. Niv et al. (2007) proposed that a major regulator of opportunity costs is the average reward rate that reflects a long-term prediction of rewards to be received. They further stated that this signal is carried by tonic dopamine levels that integrate the reward-related prediction error information reported phasic dopamine activity. Therefore, if there are

indications of, or actual unexpected rewards phasic dopamine signalling occurings, the average reward rate increases over time resulting in increased opportunity costs and heightened response vigour. Cools et al. (2011) extrapolate this mechanism to 5-HT, assuming that it has the opposite role and reports the effect of punishments on the opportunity costs. Thus, if actions performed over time result in more aversive (or punishing) outcomes, phasic 5-HT is released repeatedly to report several punishment-related prediction errors, which are, in turn, integrated into slowly changing tonic 5-HT levels to carry an average punishment rate. Cools et al. (2011) suggest that the average punishment rate has the opposite effect on the opportunity costs, which is that a high average punishment rate (reflected by high tonic 5-HT levels) reduces the opportunity costs of time and, as a result, response vigour. What follows from these conclusions is that the opportunity costs of time are controlled by both the average reward and average punishment rate. More specifically, the difference between these average reward and punishment outcome rates, the *overall average outcome*, controls the opportunity costs and, therefore, how vigorously actions are performed.

This framework demonstrates an elegant integration of the positive and invigorating aspects of dopamine and the negative and inhibitory aspects of 5-HT along with their interaction to guide behaviour via the opportunity costs of time. Moreover, although the framework is heavily influenced by the reinforcement learning paradigm, it can be readily translated to predicting behaviour in VBDM tasks, such as ITeCh or risky choice, offering a strong foundation to derive and test hypotheses about behaviour for high/low tonic 5-HT levels.

1.10. Research questions

The primary aim of this thesis was to comprehensively test the effects of 5-HT in light of the computational model by Cools et al. (2011) using four VBDM tasks that are prominent in the behavioural economics field, namely an intertemporal choice (ITeCh) task, a risky choice task for gains and losses (PDG/PDL), and a mixed-gambles (MGA) task, all taken from our VBDM battery (Pooseh, Bernhardt, Guevara, Huys, & Smolka, 2018). In order to manipulate tonic 5-HT levels in the brain, we used the well-established dietary acute tryptophan intervention method that enables tryptophan to be boosted or depleted resulting in increased or decreased tonic 5-HT levels (Moja et al., 1988; Young, 2013).

Furthermore, we investigated the serotonin transporter linked polymorphic region (*5-HTTLPR*), a genetic variation in the promoter region of the gene (SLC6A4) that codes for the SERT. This

variation regulates the gene's transcription activity: the short (S-) allele is associated with lower transcriptional activity while the long (L-) allele is connoted with higher activity (Lesch et al., 1996). The tentative conclusion is that individuals homozygous for the S-allele have higher tonic 5-HT levels (due to lower SERT expression) with converse holding true for individuals homozygous for the L-allele. This makes the genotype a prime candidate to associate with decision-making behaviour. Moreover, previous research has shown that the genotype modulates behaviour during tryptophan interventions (Marsh et al., 2006; Roiser, Muller, Clark, & Sahakian, 2007). Therefore, it is reasonable to control for genotype and explore potential interactions between genotype and tryptophan interventions.

Another aim of this thesis was to investigate the brain network associated with amount and delay processing during ITeCh and whether changes in tonic 5-HT levels are associated with changes in brain activity. To this end, we employed a modified ITeCh task for fMRI that allowed us to disentangle amount and delay information and an event-related design to measure the blood oxygen level dependent (BOLD) signal as a marker for neuronal activity.

In detail, in the genetic part of *Study 1*, we investigated associations of the three *5-HTTLPR* (two homozygous and one heterozygous) groups with probability discounting for gains and losses, as well as loss aversion. In the pharmacological part, we used an acute tryptophan intervention and three conditions (depletion, loading, and a balanced control condition) with the same tasks being used to test the prediction that increased tonic 5-HT levels reduce probability discounting for gains and losses while decreasing loss aversion. The opposite behavior was expected for reduced tonic 5-HT levels.

Study 2 was dedicated to ITeCh and also included a genetic excursion to investigate associations of *5-HTTLPR* with temporal discounting and a pharmacological part relating to the tryptophan interventions. Here, we predicted that high tonic 5-HT levels result in reduced discounting rates with the converse holding for low tonic 5-HT levels. Furthermore, on the brain level, we assumed that high 5-HT levels have a stronger positive BOLD response for amount magnitude of the delayed offer and a weaker deactivation for higher delays in the respective brain networks.

Based on the findings from *Study 1*, we used diffusion-weighted (DWI) imaging in *Study 3* to investigate whether the genotype specific group differences observed in the probability discounting rates for losses can be explained by structural differences in white-matter due to the known involvement of 5-HT in neuronal development and plasticity. We assumed reduced

structural connectivity in S/S compared to L/L carriers in two *a priori* fibre bundles, as well as negative connectivity between structural connectivity and discounting rates.

2. Study 1 – Risk-seeking for losses is associated with *5-HTTLPR*, but not with transient changes in 5-HT levels

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Abstract

Rationale: Serotonin (5-HT) plays a key role in different aspects of value-based decision-making. A recent framework proposed that tonic 5-HT (together with dopamine, DA) code future average reward expectations, providing a baseline against which possible choice outcomes are compared to guide decision-making. *Objectives:* To test whether high 5-HT levels decrease loss aversion, risk seeking for gains, and risk seeking for losses. *Methods:* In a first session 611 participants were genotyped for *5-HTTLPR* and performed a mixed gambles (MGA) task and two probability discounting tasks for gains and losses respectively (PDG/PDL). Afterwards, a subsample of 105 participants (44 with S/S, 6 with S/L, 55 with L/L genotype) completed the pharmacological study using a cross-over-design with tryptophan depletion (ATD), loading (ATL) and balanced (BAL) conditions. The same decision constructs were assessed. *Results:* We found increased risk seeking for losses in S/S compared to L/L individuals at the first visit ($p = 0.002$). Neither tryptophan depletion nor loading affected decision-making, nor did we observe an interaction between intervention and *5-HTTLPR* genotype. *Conclusion:* Our data do not support the idea that transient changes of tonic 5-HT affect value-based decision-making. We provide evidence for an association of *5-HTTLPR* with risk-seeking for losses, independent of acute 5-HT levels. This indicates that the association of *5-HTTLPR* and risk seeking for losses is mediated via other mechanisms, possibly by differences in the structural development of neural circuits of the 5-HT system during early life phases.

2.1. Introduction

The neuromodulator serotonin (5-hydroxytriptamine, 5-HT) has been found to play an important role in different aspects of value-based decision-making (VBDM), although the mechanisms by which it exerts its influence have yet to be fully resolved (Faulkner & Deakin, 2014; Mendelsohn, Riedel, & Sambeth, 2009). Based on experimental findings in animals and humans, as well as reinforcement learning theories which provide evidence about how rewards and punishments improve predictions about long-term (average) outcomes, it has been suggested that dopamine (DA) and 5-HT are candidates to carry reward and punishment signals, respectively. More precisely, central tonic DA levels (in contrast to phasic DA levels) may signal the average reward rate over a time period one can expect from a series of outcomes and tonic 5-HT levels signal the average punishment rate that is associated with this series. Furthermore, these tonic DA and 5-HT levels may interact with each other to establish an overall average outcome rate and that this interaction can be formalized as the difference between average reward rate and the average punishment rate, i.e. the tonic DA and 5-HT levels, respectively. This rate can be conceptualized as a sort of baseline against which potential outcomes are then compared and determines whether an outcome is perceived as a net gain or loss (Cools et al., 2011; Daw et al., 2002; Niv et al., 2007).

This framework by Cools et al. (2011) can be applied to a variety of decision-making dilemmas, including risk. In this domain, it is well known that humans do not make risky choices related to the final outcome, but rather in relation to a reference point (Kahneman & Tversky, 1979). This reference point may be represented by the overall average outcome rate, which is determined by tonic DA and 5-HT levels. For example, decreasing 5-HT levels (assuming constant DA levels) in light of a small sure and a large probabilistic gain with previously equal subjective value should increase the reference point. This would decrease the perceived amount of both offers by a fixed amount, which makes the small sure gain loom like a small loss and the large probabilistic gain like a small gain. Thus, the probabilistic gain, compared to the sure small loss is perceived as more attractive, hereby increasing risk seeking for gains. In the same way, when confronted with choices that involve a small certain loss and a larger but probabilistic loss, increased 5-HT (i.e. a decreased reference point) should decrease risk-seeking for losses and shift choice preference towards the small but certain loss. The reason is that small losses now appear as small gains that are more attractive than a probabilistic loss, although the latter also appears to be reduced. For tasks that incorporate options for either taking

a mixed gamble that includes both the prospect to win and to lose or not to gamble, high 5-HT levels (decreased reward expectations) should reduce the impact of a potential loss but increase the value of a potential gain, and therefore reduce loss aversion.

A non-invasive way to pharmacologically manipulate 5-HT synthesis in the brain is to administer amino acid mixtures that either lack tryptophan, the precursor amino acid for 5-HT synthesis (acute tryptophan depletion, ATD) or contains a higher amount of tryptophan (acute tryptophan loading, ATL) assuming that a decrease or increase of tryptophan levels changes 5-HT levels in the brain accordingly. The effectiveness of ATD and ATL on brain 5-HT synthesis has been shown in several animal (Biskup et al., 2012; Tagliamonte, Biggio, Vargiu, & Gessa, 1973; Tagliamonte, Tagliamonte, Perez-Cruet, & Gessa, 1971) and human studies (Carpenter et al., 1998; Moja et al., 1988; Nishizawa et al., 1997; Williams, Shoaf, Hommer, Rawlings, & Linnoila, 1999).

In general, the framework described by Cools et al. (2011) detailed above has received some support from animal and human studies that assessed the impact of reduced and/or increased 5-HT levels. For example, according to this framework, reduced 5-HT should increase the baseline (i.e. higher overall outcome expectation), thus making waiting more costly when immediate gains are available, and indeed reduced 5-HT levels were associated with increased temporal discounting rates in several studies (Mobini, Chiang, Ho, Bradshaw, & Szabadi, 2000; Schweighofer et al., 2008; Wogar, Bradshaw, & Szabadi, 1993). The opposite, increased 5-HT levels reduced temporal discounting rates, has also been observed (J. C. Bizot, Thiebot, Le Bihan, Soubrie, & Simon, 1988; Poulos, Parker, & Le, 1996). Regarding probability discounting of gains, reduced 5-HT levels via ATD, increased choices for probabilistic gains compared to sure but smaller gains, hence reduced risk-aversion in macaques (Long, Kuhn, & Platt, 2009). A similar observation congruent with the predictions of the framework was reported in another ATD study, which found that depleted rats chose larger but probabilistic food rewards instead of smaller but certain food rewards (Koot et al., 2012). Two studies investigated probability discounting in the loss domain: S. E. Murphy et al. (2009) reported that participants showed reduced risk-seeking for losses after receiving a 14 day diet with tryptophan supplements and Rogers et al. (2003) who used ATD and a sham depletion found no effect of ATD on risk-seeking for losses. Finally, there is some evidence whether TRP interventions affect gambling behaviour with mixed prospects. Rogers et al. (2003) observed in trials in which participants had to choose between a control gamble and varying experimental gambles (both gambles involved the prospect to win or lose), that ATD reduced discrimination

between magnitudes of expected gains, independent of the magnitude of losses and the probability to receive them. This finding is at odds with the Cools et al. (2011) framework because ATD should increase the reference point to which outcomes are compared and thus promote choices for the high magnitude gain (i.e. better discrimination).

Rather indirect evidence comes from a study that used a probabilistic reversal learning task. Chamberlain et al. (2006) reported increased inappropriate switching following negative feedback after a single dose of the selective serotonin reuptake inhibitor citalopram, which is believed to decrease central 5-HT levels by acting at inhibitory 5-HT1A (Blier, Serrano, & Scatton, 1990; Selvaraj et al., 2012) and can therefore be interpreted as increased loss sensitivity in line with the framework. However, it should be noted that it is not yet clear whether the primary mechanism of a single dose of SSRI is a net reduction in tonic 5-HT levels, which makes it difficult to interpret the results in such a way. Therefore, in our study, we applied an acute tryptophan intervention as a more straightforward way to manipulate tryptophan availability and 5-HT synthesis in the brain (Biskup et al., 2012) and to comprehensively test the predictions of the Cools et al. (2011) framework with a recently developed value-based decision-making battery. In this way, we are able to cover multiple facets of decision-making behaviour with a sample size that is two to three times higher than in previous studies, which assessed between 20 and 40 participants (i.e. S. E. Murphy et al., 2009; Rogers et al., 1999; Rogers et al., 2003; Schweighofer et al., 2008; Talbot, Watson, Barrett, & Cooper, 2006), thus avoiding potential methodological issues.

Furthermore, another important modulator of 5-HT function is a common polymorphism in the promotor region of the SLC6A4 gene that codes for the 5-HT transporter (5-HTT). This region, known as the *5-HTTLPR* (5-HTT gene-linked polymorphic region) has been studied extensively because the availability of 5-HTT is crucial for the concentration of extracellular 5-HT (Lau & Schloss, 2012). The *5-HTTLPR* is a repeat polymorphism where the 14 repeat allele is referred to as the short (S) allele and the 16 repeat allele as the long (L) -allele (Heils et al., 1996). The short allele has been associated with reduced transcriptional activity, reduced 5-HTT expression and increased extracellular 5-HT concentration in several cell line experiments and mouse models (reviewed in D. L. Murphy, Lerner, Rudnick, & Lesch, 2004; Tripp & Sibille, 2010). The main interest in *5-HTTLPR* evolved after the initial finding that the S-allele was associated with higher trait anxiety and neuroticism (Lesch et al., 1996), suicide (Courtet et al., 2004), and disorders related to 5-HT function such as depression (Hoefgen et al., 2005), possibly by complex interactions with the environment (Caspi et al., 2003; van der Meer et al., 2015). In

humans, it is difficult to directly study the relationship between *5-HTTLPR* and 5-HT in the brain in vivo. Therefore, imaging techniques such as positron emission tomography (PET) or single photon emission computed tomography (SPECT) have been employed to shed light on how *5-HTTLPR* influences 5-HTT density in the human brain. There is some evidence that *5-HTTLPR* has an impact on 5-HTT availability, for example, using [123I]β-CIT Heinz et al. (2000) showed that L/L individuals have higher binding potential in the raphe area and other studies that used [11C]DASB found higher binding potential in midbrain, putamen and in the caudate nucleus (Kalbitzer et al., 2009; Praschak-Rieder et al., 2007; Reimold et al., 2007) compared to S-carriers. Although the exact mechanism of how *5-HTTLPR* influences 5-HT levels is not well understood, we assume that the S/S genotype is associated with increased 5-HT levels due to a reduced amount of 5-HT transporters to reuptake 5-HT (Heils et al., 1996; Mathews et al., 2004). It should be noted that a contrasting view exists based on the ameliorating effect of selective serotonin inhibitors (SSRI), which increase 5-HT, on depression (Arroll et al. 2005; but see Andrews et al. 2015).

Moreover, several reports have established an association between *5-HTTLPR* variation and decision-making strategies. A popular task is the Iowa Gambling Task (IGT), which offers participants to choose a total of 100 cards from four card decks of which two offer high gains but even higher losses in the long run (hence termed disadvantageous) and the other two offer small gains but even smaller losses in the long run (hence termed advantageous). Three studies showed that the S-allele was associated with more choices from the disadvantageous decks (He et al., 2010; Homberg, van den Bos, den Heijer, Suer, & Cuppen, 2008; R. van den Bos, Homberg, Gijsbers, den Heijer, & Cuppen, 2009) but one more recent study found better performance for S-allele carrier, possibly related to a higher susceptibility to the risk when learning the task (Miu et al., 2012). This explanation is in agreement with findings in other tasks that assess risky choice behaviour, where S-allele carriers showed more risk-aversion under ambiguity, i.e. in a Balloon Analogue Risk Task (Crisan et al., 2009) and invested less money in risky assets in an investment task, a finding that has been replicated (Kuhnen & Chiao, 2009; Kuhnen, Samanez-Larkin, & Knutson, 2013). In a gambling task, Heitland et al. (2012) reported that S-allele carriers were more risk-averse after winning money in the previous trial compared to L/L carriers. The increased sensitivity towards risk is in agreement with the framework by Cools et al. (2011), because the S-allele carriers behaved as if they have increased tonic 5-HT levels, which would reduce the reference point, making sure gains more attractive in comparison to the risky option. However, one study, which made participants choose between

smaller or larger losses in a reward/punishment task, did not find an effect of genotype (Blair et al., 2008). So far, no study specifically investigated the impact of *5-HTTLPR* on risk-seeking for losses using smaller sure and larger probabilistic losses. For loss aversion, as measured with a mixed gambles task, the data are inconclusive. He et al. (2010) reported increased loss aversion (i.e. rejecting more gambles) for S/S individuals, while a study by Ernst et al. (2014) found reduced loss-aversion in anxious adolescents with L/L genotype and no effect in anxious S/S adolescents. They found however, no differences genotype groups in the control group, highlighting the need for more studies to clarify these findings.

Additionally, it has been suggested that the genetic variation of *5-HTTLPR* might influence the effect of tryptophan interventions. For example, ATD had a stronger impact on L/L genotype by reducing the performance to discriminate between punishment stimuli and probabilities of winning compared to S-allele carrier (Blair et al., 2008; Finger et al., 2007) while other studies showed in contrast reduced motivational speeding in S/S individuals after ATD compared to L/L carrier and impaired processing of fearful expressions in S-allele carrier (Marsh et al., 2006). An animal study with SERT knockout rats showed that rats lacking the 5-HTT gene were most vulnerable to ATD in frontal cortex and hippocampus compared to heterozygous rats and wildtype controls which was also reflected in impaired object recognition (Olivier et al., 2008). Based on these findings, the effect of tryptophan interventions on decision-making can be modulated by *5-HTTLPR*, although the heterogeneity of findings suggests that the genetic modulation differs depending on the behavioural task. Therefore, we accounted for this potential modulatory effect in our study.

Overall, these lines of research provide a fruitful ground to directly test the predictions derived from the framework proposed by Cools et al. (2011). Accordingly, we conducted a study consisting of two parts: in a large sample (n = 611) we tested the association of *5-HTTLPR* and decision-making using three computerized tasks that measure probability discounting for gains and losses to assess risk-aversion for gains and risk-seeking for losses respectively, as well as loss aversion with a mixed-gambles task. In a subsample of participants (n = 105), we thereafter applied three dietary tryptophan interventions to acutely increase or decrease 5-HT brain levels, or to keep them constant. We predicted that increased tonic 5-HT levels, due to a presumably reduced reference point for potential outcomes, should decrease risk seeking for gains and losses, and should also decrease loss aversion. The opposite effects were expected for decreased tonic 5-HT. Furthermore, we explored whether the effects of the intervention are modulated by *5-HTTLPR*.

2.2. Materials and Methods

2.2.1. Participant Recruitment

A random sample of the Dresden population, stratified by age and gender between 20 and 40 years was drawn by the registration office. We then sent 15750 letters containing general information about the purpose of the study and the possibility to participate. 1383 individuals responded to the letters and were pre-screened by trained psychologists via telephone. Exclusion criteria were pregnancy, not fulfilling the common criteria for MR safety, a current somatic disease requiring medical treatment, any psychiatric disorders that required pharmacological treatment within the last year, a lifetime history of one of the following conditions (for ICD-10): organic psychiatric disorders (F0), opiate, cocaine, stimulants, hallucinogens, inhalants, or poly-substance dependence, schizophrenia or related personality disorders (F2), and affective disorders (F3). Participants who passed the screening were invited to the study, where additionally their visual acuity was checked to ensure that it was at least 0.8. The final sample consisted of 611 individuals who gave written informed consent. The study was approved by the local ethics committee of the Technische Universität Dresden. A graphical depiction of the recruitment workflow is shown in the supplemental material (Fig. S1).

2.2.2. Experimental procedure: genetic study

During the first visit, we sampled blood from each participant. DNA was extracted according to standard procedures. Afterwards, they were seated in muted booth at a computer where they completed several tests and questionnaires (a complete overview is available in the supplemental material, section 71.2.) and a value-based decision-making (VBDM) battery consisting of three tasks (for details see respective section below). All participants were compensated with the gains from the battery they received but at least 10 Euro/hour (average win: 27.48 Euro ± 7.80). From the 611 completed sessions, no genotype information was available for nine participants, resulting in 602 genotyped participants. Of those, seven could not be assigned to their VBDM data sets. From the remaining 595 participants, 18 had to be discarded due to completely missing data (n = 3) or missing data sets in one (n = 7) or two tasks (n = 8), leaving 577 complete data sets for analysis.

2.2.2.1. Genotyping

Genotyping of *5-HTTLPR* was conducted at the Central Institute of Mental Health in Mannheim, Germany. A detailed description of the genotyping procedure is provided elsewhere (Dukal et al., 2015). Blood from 602 participants was available for genotyping. The observed allele frequency was 39.9% for S and 60.1% for L, with the following genotype groups: 99 S/S, 283 S/L and 220 L/L. The allele and genotype frequencies did not deviate from Hardy-Weinberg equilibrium (χ^2 = 0. 2461, df = 1, p = 0.62).

The inclusion criteria for the pharmacological study were the same as described above. Originally, we planned to measure a subsample of at least 100 participants, half homozygous for the S and half homozygous for the L-allele. Since only a small proportion of participants of the genetic study consented to also participate in the pharmacological study, and high drop-out rates, we could only investigate 44 S/S subjects and therefore included 6 S/L participants. For the LL group we stopped the assessment after completing 55 subjects.

2.2.3. Experimental procedure: pharmacological study

All participants arrived at three separate days, separated at least for a week in order to ensure complete wash-out of the intervention, to be tested in a double-blind, within-subject cross-over study design. They were compensated with at least 180 Euro for all three sessions (the average win was 196 Euro ± 28). Participants arrived either at 8.30 a.m. or 10.30 a.m. at the neuroimaging centre after following a low-protein diet the day before and having fasted overnight. Blood was sampled via a peripheral venous catheter (Braunüle®) and prepared for storage (see section Biochemical Measures). Afterwards they received one of the three amino acid mixtures (details of the constituents in the next section). After ~4 hours they completed the PDG/PDL and MGA tasks at the computer. After each session, they received their payment and a snack. Of the 112 participants who completed all three sessions, five received accidently the same amino acid mixture in all sessions and had to be excluded. From the remaining 107 participants, two more were excluded because they had incomplete data for the PDL and MGA tasks due to technical issues, resulting in 105 subjects. A more detailed description of mood and behavioural assessment not reported here is described in the supplemental material, section 7.1.3.

2.2.3.1. Amino Acid Mixture

In order to minimize negative side effects of the mixture and increase tolerance we used the 'Moja-De' protocol (Dingerkus et al., 2012) which consists of fewer different amino acids compared to other protocols (Young, Smith, Pihl, & Ervin, 1985) and takes body weight into account. All mixtures contained the same amount of large neutral amino acids (LNAA) but differed in the amount of tryptophan. In the depletion condition (ATD) tryptophan was completely absent; the balanced condition (BAL) contained 7 mg/kg body weight and the loading condition (ATL) contained 70 mg/kg body weight. The LNAAs had the same quantity for every participant across sessions (dosages per kg body weight): L-phenylalanine (132 mg), L-leucine (132 mg), L-isoleucine (84 mg), L-methionine (50 mg), L-valine (96 mg), L-threonine (60 mg) and L-lysine (96 mg). The mixtures were prepared by a commercial manufacturer (Amino-Factory, 88161 Lindenberg, Germany).

2.2.3.2. Biochemical Measures

In order to assay plasma tryptophan and LNAA levels, venous blood was sampled in EDTA tubes (Sarstedt, Germany) on each study day at four time points during a session: at the beginning of the session as reference (T0), one hour after drinking the mixture (T1), ~3 hours after drinking the mixture (T2) and finally shortly before the end of the session (~6 hours after drinking the mixture, T3). Collected samples were immediately centrifuged for 10 minutes at 4000g, 4°C. Plasma was then stored at -81°C. Analyses were conducted at the Department of Chemistry and Food Chemistry of the Technische Universität Dresden as described previously (Henle, Walter, Krause, & Klostermeyer, 1991).

2.2.4. Value-Based Decision-Making Battery (VBDM)

The VBDM tasks included probability discounting for gains (PDG), losses (PDL) and mixed-gambles (MGA), which measure different facets of value-based decision-making. In each of the tasks, pairs of monetary offers were presented sequentially in a binary choice format and participants chose one of two simultaneously presented offers according to their preference. All offers were presented randomly shown on the left or right side on the computer screen and the chosen offer was indicated with a red frame. Participant were informed beforehand that one of their choices in every task would be selected randomly and being paid. During the tasks, they did not get feedback about the outcomes of each choice. Several meta-control parameters such as discounting rates (k) were estimated for PDG/PDL (a measurement of risk-aversion for gains

and risk-seeking for losses respectively) and loss aversion (λ) for MGA. For PDG and PDL a hyperbolic discounting was assumed following the well-known formula:

$$V = \frac{A}{(1+k\Theta)}$$

where Θ = (1 – P)/P is the transformation of reward probability P (2/3, 1/2, 1/3, 1/4, and 1/5) to odds against winning. The amount, A, ranges from 5 Euro to 30 Euro for PDG and -5 Euro to -30 Euro for PDL. To estimate loss aversion for the MGA tasks the following value function was used:

$$V = \frac{(G - \lambda L)}{2}$$

where L is the loss magnitude, and G the gain magnitude. The amount range for gains was 1 Euro to 40 Euro and the losses ranged from -5 Euro to -20 Euro. At the beginning of the task, participants received 10 Euro of "house money". The PDG/PDL tasks consisted of 30 trials and the MGA task of 40 trials. The estimated *k* and λ parameters from the VBDM best explain individual choice behaviour with high *k* values in the PDG task indicating risk aversion (i.e. discounting of probabilistic wins) and high *k* values in the PDL task indicating risk seeking by discounting higher but probabilistic losses. Finally, high λ values represent higher loss aversion and therefore less gambling behaviour in the MG task.

In all tasks, the likelihood of choosing between the two offers follows a softmax probability function in which $\beta > 0$ serves as a consistency parameter such that its large values correspond to a high probability of taking the most valuable action. The algorithm starts from liberal prior distributions on the parameters and, after observing a choice at each trial, updates the belief about the parameters using the Bayes' rule $P(k, \beta | choice) \propto P(choice | k, \beta) P(k, \beta)$. We assume normally distributed priors with mean values in the middle of the parameters' range ($-10 \leq k \leq 10$, $-8 \leq \lambda \leq 2$, $-8 \leq \beta \leq 8$) and large variances ($\sigma^2 = 30$) to avoid strong initial biases. The procedure continues for 30 (40 in Mixed Gambles) trials to reach a stable estimation. The estimates at the final trial are considered as the best fitting parameters for a participant. A more detailed description of the mathematical framework has been reported elsewhere (Pooseh et al., 2018). All tasks were implemented in MATLAB (Release 2010a, The MathWorks, Inc., Natick, Massachusetts, United States) and the Psychtoolbox 3.0.10 based on the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997).

2.2.5. Statistical analyses

All statistical analyses were done in SPSS 23 (IBM-SPSS, Chicago, IL, United States). For the analysis of the data, the log-transformed parameters were used because they approach a normal distribution.

To test for association between genotype and decision-making all three parameters (*k* from PDG and PDL, λ from MGA) were entered into a multivariate analysis of variance (MANOVA) with *5-HTTLPR* (S/S, S/L, LL) as a between-subject factor. Additionally, gender was included as a control variable. If there was neither a main effect of gender nor an interaction with genotype, gender was removed from the model. The MANOVA analysis was based on 577 participants who had complete data for all tasks. In a next step to improve the estimation of confidence intervals, data were resampled with 10.000 iterations. Significance was assumed at $p < .05$. Subsequent post-hoc t-tests corrected for unequal variances were conducted when a significant result occurred.

To estimate the pharmacokinetic effect of the interventions we computed free plasma tryptophan levels and the ratio of tryptophan plasma levels to the sum of the other large neutral amino acids (∑LNAA). It should be noted that although we measured free tryptophan, we based our inferential statistics on the ratio parameter because it is physiologically more informative (Fernstrom & Wurtman, 1972). To account for inter-individual differences in baseline blood levels, all four time points were normalized by subtracting the reference measure (T0) from the other time points (T1, T2, T3). We integrated over all time points to compute area under the curve (AUC) scores, which were then entered into repeated-measures ANOVA as dependent variable with intervention as a within-subjects factor and gender as a control variable because previous literature has shown that this variable may influence the effect of the intervention. At the time of the analyses, blood plasma level data from 92 participants were available. Of those, one had to be discarded due to a processing error of the blood plasma leaving 91 valid data sets. Because we subtracted the reference measure (T0) from T1, T2, and T3 it was possible to have negative AUC values which indicates a decrease in peripheral blood measures with respect to T0.

To test the a priori hypothesized main effect of the tryptophan intervention (ATL > ATD) we first entered VBDM parameters into a planned simple linear contrast analysis. To this end, we calculated difference scores for each task by subtracting the ATD from the ATL condition. We then conducted one-sample t-tests to test whether the difference scores were significantly

different from zero. Additionally, we employed a resampling procedure with 10.000 iterations to improve the estimation of confidence intervals. Second, we conducted exploratory analyses to look for potential non-linear effect of the interventions (e.g. increasing and decreasing tonic 5-HT levels might have the same effect, or only one intervention has an effect) and interaction effects with *5-HTTLPR*. To this end, we computed three separate repeated-measures analyses of variance (rm-ANOVA) one for the outcome of each task as dependent variable, with intervention (ATD, BAL, ATL) as a within-subject factor and genotype (S/S, S/L, L/L) as a between-subject factor. Third, we computed three additional rm-ANOVAs with intervention as within-subject and gender and intervention order as between-subject factors to explore main effects of gender and intervention order and their interaction with the factor intervention.

2.3. Results

Demographic information of all participants is shown in Table 1. Genotype groups did not significantly differ with respect to gender and age, neither in the genetic, nor in the pharmacological study.

Table 1 Demographic information

	N (female)	Age (SD)
Genetic study		
S/S	96 (41)	31.92 (6.0)
S/L	272 (130)	32.60 (5.5)
L/L	209 (95)	33.14 (5.5)
Statistic	$\chi_2^2 = .794$, ns.	$F_{2,574} = 1.67$, ns.
Pharmacological study		
S/S	44 (13)	32.25 (5.8)
S/L	6 (2)	33.00 (7.8)
L/L	55 (25)	31.89 (6.0)
Statistic	$\chi_2^2 = 2.685$, ns	$F_{102} = .11$, ns.

2.3.1. Genetic study

2.3.1.1. Association between 5-HTTLPR and value-based decision-making

The multivariate analysis of variance (MANOVA) demonstrated no overall difference between *5-HTTLPR* genotypes across tasks (Wilk's Lambda = .985, p = .187, Cohen's d = .18). However, as shown in the first row of Fig. 1, there was a strong univariate significant association of *5-HTTLPR* genotype and risk seeking for losses as assessed with PDL ($F_{2,571}$ = 3.594, p = .028, Cohen's d = .22). A Games-Howell post hoc test showed that participants with S/S genotype were more risk seeking for losses compared to those with S/L genotype (p = .018, BCa 95% CI [.0639 .6380], Cohen's d = .23) and L/L genotype (p = .001, BCa CI [.2037 .8286], Cohen's d = .33). There was no significant difference between S/L and L/L genotype (p = .294, BCa CI [-.1353 .4703], Cohen's d = .10).

There were no significant genotype effects neither on risk aversion for probabilistic gains (PDG, $F_{2,571}$ = .602, p = .548, Cohen's d = .09) nor on loss aversion as measured with MGA ($F_{2,571}$ = .241, p = .786, Cohen's d = .06). Finally, there were no significant main or interaction effects of gender for any VBDM task (all p > .05). All scores from the VBDM battery for the genetic study can be found in Table 2.

2.3.2. Pharmacological study

2.3.2.1. TRP/∑LNAA peripheral blood plasma levels

As can be seen in Fig. 2, tryptophan levels peaked after 3 h after loading and reached a minimum after 3 h following depletion, and returned to baseline levels after 6 h in both conditions. Tryptophan/Sum of large neutral amino acids (TRP/∑LNAA) values showed as expected the highest TRP/∑LNAA values for ATL, intermediate values for BAL and the lowest values for ATD. Descriptive data can be found in Table 3. The repeated measures ANOVA revealed a significant main effect of intervention on TRP/∑LNAA AUC scores ($F_{1.1, 95.4}$ = 621.986, p < 4.8 x 10^{-81}). A contrast analysis showed significant differences in TRP/∑LNAA AUC scores between ATD and BAL ($F_{1,89}$ = 186.356, p < 1.5 x 10^{-23}), as well as between BAL and ATL ($F_{1,89}$ = 573.556, p < 1.4 x 10^{-40}). There were neither main effects nor interactions for gender (all p > .20).

Table 2 Genetic study: VBDM scores by *5-HTTLPR*

	S/S		S/L		L/L	
Measure	Md (IQR)	N	Md (IQR)	N	Md (IQR)	N
Males						
PDG (*k*)	0.95 (1.14)	55	1.02 (0.85)	142	0.93 (0.93)	114
PDL (*k*)	0.86 (0.77)	55	0.87 (0.73)	142	0.82 (0.68)	114
MGA (λ)	1.16 (1.26)	55	1.09 (0.91)	142	1.03 (0.98)	114
Females						
PDG (*k*)	1.34 (1.42)	41	0.80 (1.01)	130	0.95 (0.96)	95
PDL (*k*)	0.82 (0.72)	41	0.64 (1.01)	130	0.68 (0.99)	95
MGA (λ)	1.14 (0.99)	41	1.05 (0.85)	130	1.08 (1.57)	95
Total						
PDG (*k*)	0.98 (1.30)	96	0.97 (0.92)	272	0.93 (0.94)	209
PDL (*k*)	0.85 (0.74)	96	0.76 (0.86)	272	0.77 (0.77)	209
MGA (λ)	1.15 (1.17)	96	1.08 (0.86)	272	1.06 (1.21)	209

PDG, Probability Discounting Gains; PDL, Probability Discounting Losses; MGA, Mixed Gambles; Md, Median; IQR, Interquartile Range. Data not log-transformed.

2.3.2.2. Effects of intervention on value-based decision-making

Overall, there was no main effect of intervention on the VBDM parameters as shown in the second row of Fig. 1. More specifically, planned simple linear contrast analyses showed no main effect of intervention on risk aversion for gains (PDG, t_{104} = -1.781, p = .074, BCa CI [-.3332 .0090], Cohen's d = -.17), nor on risk seeking for losses (PDL, t_{104} = -.807, p = .424, BCa CI [-.4321 .1770], Cohen's d = -.08), nor on loss aversion (MGA, t_{104} = .498, p = .627, BCa CI [-.0948 .1706], Cohen's d = .05).

Moreover, the exploratory rm-ANOVAs did not reveal significant differences in any of the three VBDM scores between any of the three interventions (e.g. increasing and decreasing tonic 5-HT levels might have the same effect, or only one intervention has an effect) and also confirmed the null finding from the contrast analyses (PDG, $F_{2, 204}$ = .361, p = .698, η^2 = .004; PDL, $F_{1.8, 180.2}$ = .274, p = .733, η^2 = .003; MGA, $F_{1.9, 190}$ = .352, p = .688, η^2 = .003). In addition, there was no significant interaction of intervention and genotype, excluding the possibility that the intervention might have had opposing effects in both genotype groups, or effects restricted to one genotype group, which might have masked the intervention effect (PDG, $F_{4, 204}$ = .292, p = .883, η^2 = .006; PDL, $F_{3.5, 180.2}$ = .721, p = .562, η^2 = .014; MGA, $F_{3.7, 190}$ = .479, p = .738, η^2 = .009). There were also no main effects of genotype (PDG, $F_{2,102}$ = .245, p = .783, η^2 = .005; PDL, $F_{2,102}$ = 1.802, p = .170, η^2 = .034; MGA, $F_{2,102}$ = .139, p = .870, η^2 = .003). Assigning the S/L genotype subjects to either the S/S or L/L group, or excluding them from the analysis did not change the results (supplemental material, Fig. S2).

Finally, the rm-ANOVAs showed no main effect of neither gender nor of intervention order (all p > .06), nor interaction effects (all p > .1). All VBDM scores from the main study can be found in Table 4.

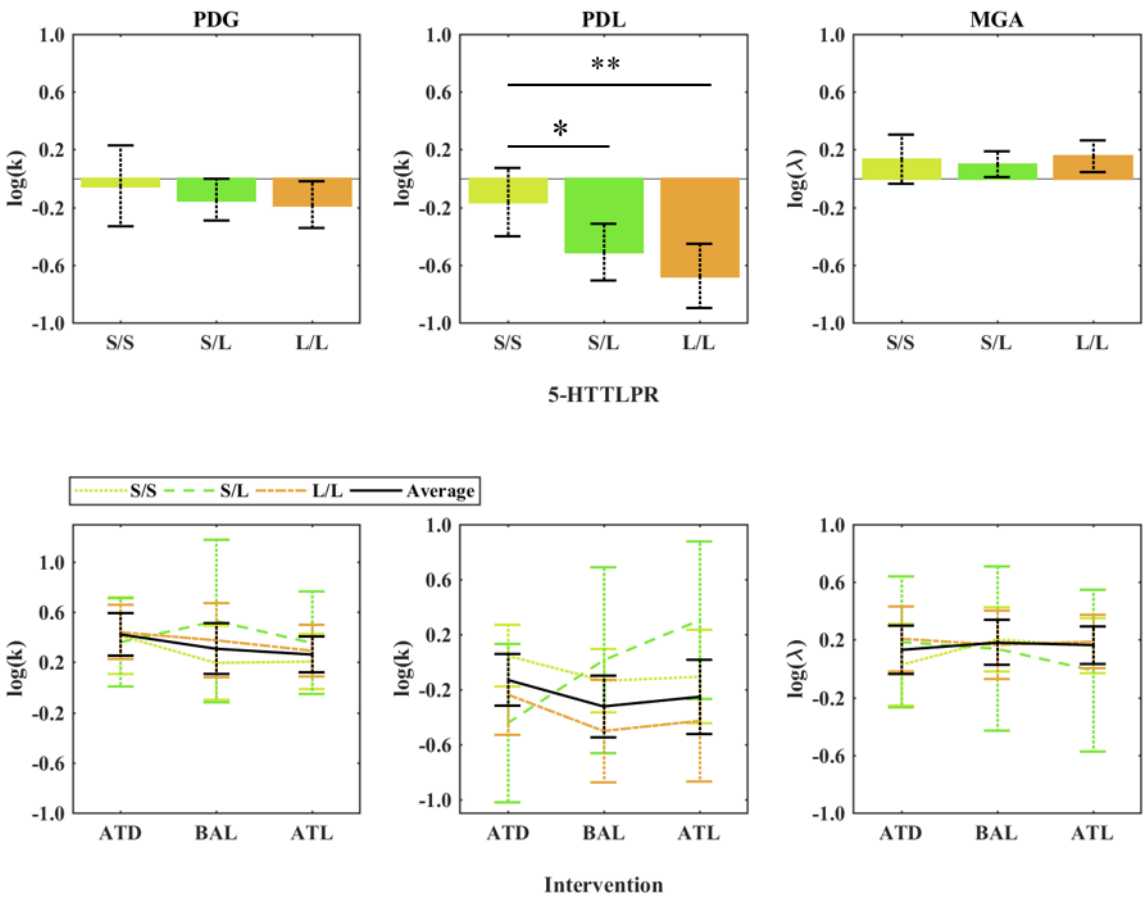

Fig. 1 Effect of *5-HTTLPR* and intervention on three meta-control parameters derived from the VBDM battery tasks. The first row shows the data collected in the genetic study for the biallelic *5-HTTLPR* genotypes and the second row shows the data across the different acute tryptophan intervention conditions: depletion (ATD), balanced (BAL) and loading (ATL). Left column: Probability discounting rate k for gains. Middle column: Probability discounting rate k for losses. Right column: Loss aversion λ. Mean scores and confidence intervals are log scaled and bootstrapped with 10.000 iterations. The error bars indicate 95% bias corrected and accelerated confidence intervals. *p < .05, **p < .01.

Table 3 Blood plasma tryptophan measures

	TRP	TRP/∑LNAA (%)	N
ATD			
T0	32.85 (7.23)	3.79 (0.61)	91
T1	29.61 (7.75)	1.53 (0.50)	91
T2	22.45 (9.24)	1.13 (0.58)	91
T3	22.81 (8.75)	1.73 (0.74)	91
AUC	-45.84 (25.64)	-13.16 (2.45)	91
BAL			
T0	33.64 (7.90)	3.91 (0.75)	91
T1	45.25 (13.79)	2.33 (0.70)	91
T2	40.23 (12.09)	2.01 (0.63)	91
T3	32.76 (9.66)	2.53 (0.78)	91
AUC	28.98 (26.38)	-9.39 (2.18)	91
ATL			
T0	33.51 (7.81)	3.83 (0.61)	91
T1	129.23 (39.14)	7.31 (1.78)	91
T2	150.21 (47.33)	8.35 (2.27)	91
T3	84.82 (39.86)	6.78 (2.73)	91
AUC	516.37 (199.58)	21.10 (11.20)	91

TRP = Free tryptophan [µmol/L]; ∑LNAA = sum of large neutral amino acids; Mean (SD)

Table 4 Pharmacological study: VBDM scores by *5-HTTLPR* and intervention

	S/S				S/L				L/L			
	ATD	BAL	ATL		ATD	BAL	ATL		ATD	BAL	ATL	
	Md	Md	Md		Md	Md	Md		Md	Md	Md	
Measure	(IQR)	(IQR)	(IQR)	N	(IQR)	(IQR)	(IQR)	N	(IQR)	(IQR)	(IQR)	N
Males												
PDG (*k*)	1.36	1.34	1.39	31	1.77	1.55	1.20	4	1.34	1.04	1.10	30
	(0.98)	(2.17)	(0.87)		(0.59)	(2.44)	(0.97)		(1.21)	(1.31)	(0.87)	
PDL (*k*)	1.12	0.91	1.10	31	1.19	0.74	1.25	4	1.00	0.89	0.92	30
	(0.84)	(1.05)	(0.83)		(1.15)	(1.22)	(0.83)		(0.96)	(0.92)	(0.72)	
MGA (λ)	1.41	1.30	1.17	31	0.79	0.95	0.77	4	1.17	1.05	1.12	30
	(1.59)	(1.18)	(1.29)		(1.13)	(0.83)	(1.59)		(1.42)	(0.99)	(1.58)	
Females												
PDG (*k*)	2.22	1.26	1.06	13	1.19	3.38	2.08	2	1.82	2.05	1.46	25
	(2.51)	(2.19)	(1.28)		(nc)	(nc)	(nc)		(2.35)	(2.67)	(1.26)	
PDL (*k*)	1.29	0.92	1.23	13	0.34	2.29	2.73	2	0.58	0.86	0.80	25
	(1.18)	(1.69)	(0.57)		(nc)	(nc)	(nc)		(0.84)	(1.35)	(1.30)	
MGA (λ)	0.88	0.97	1.04	13	2.14	2.32	1.64	2	1.23	1.33	1.17	25
	(0.61)	(0.98)	(0.75)		(nc)	(nc)	(nc)		(1.43)	(1.25)	(0.73)	
Total												
PDG (*k*)	1.42	1.30	1.23	44	1.71	1.55	1.20	6	1.53	1.15	1.29	55
	(1.65)	(2.14)	(0.92)		(0.79)	(3.12)	(1.59)		(1.42)	(2.10)	(1.12)	
PDL (*k*)	1.15	0.91	1.15	44	0.66	1.16	1.37	6	0.88	0.86	0.90	55
	(0.90)	(1.04)	(0.86)		(1.20)	(1.53)	(1.72)		(0.89)	(0.98)	(0.98)	
MGA (λ)	0.97	1.12	1.09	44	1.23	0.95	0.83	6	1.22	1.13	1.16	55
	(1.10)	(1.18)	(1.05)		(1.55)	(1.68)	(1.91)		(1.36)	(0.97)	(0.97)	

PDG, Probability Discounting Gains; PDL, Probability Discounting Losses; MGA, Mixed Gambles; ‘nc‘, not computable; Md, Median; IQR, Interquartile Range. Data not log-transformed.

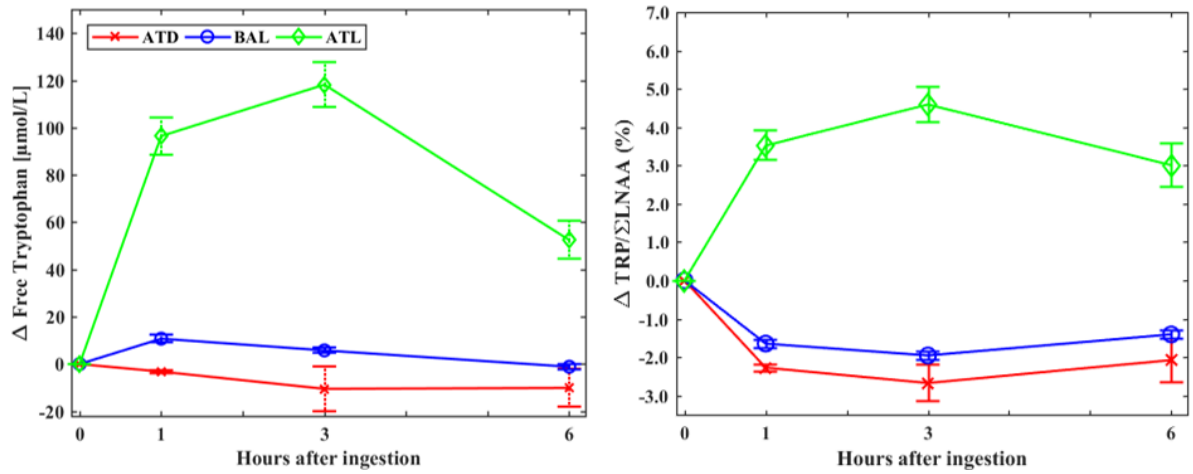

Fig. 2 Pharmacokinetics of the acute tryptophan intervention (N = 91). The baseline measure (T0) was subtracted from the other time points to normalize the curves with respect to inter-individual differences in plasma levels. The left graph shows the plasma levels of free tryptophan. The right graph shows the ratio of tryptophan to the other large neutral amino acids. The abbreviations are acute tryptophan depletion (ATD), balanced tryptophan (BAL) and acute tryptophan loading (ATL). The shaded areas indicate bias corrected and accelerated (BCa) 95% confidence intervals (CI). Mean scores and CIs are based on 10.000 bootstrapping iterations.

2.4. Discussion

To the best of our knowledge, this is the first study that extensively tested the predictions of the framework by Cools et al. (2011), which assumes that tonic 5-HT levels (together with tonic DA levels) control a reference point against which all outcomes, gains as well as losses, are compared. To this end, we assessed three different parameters of value-based decision-making in a community sample stratified by *5-HTTLPR* genotype. According to the model's predictions, we expected increased tonic 5-HT levels to lower the reference point and hence reduce risk-seeking for probabilistic gains as well as losses, and to also reduce loss aversion. In contrast to our hypotheses, we did not find any significant effect of the interventions (ATD as well as ATL) on any of the three decision-making parameters. Nevertheless, with respect to genotype, we found an association of *5-HTTLPR* with risk seeking for losses. S/S carriers were more risk seeking for losses compared to S/L and L/L carriers. No other associations of *5-HTTLPR* were observed.

2.4.1. Pharmacological study

In humans, the empirical evidence for the effect of 5-HT on value-based decision-making is so far inconclusive. A study by I. M. Anderson, Richell, and Bradshaw (2003) used a wheel-of-fortune task where wheel A always had a fixed probability of ~95% (17/18) winning and varying amounts from 10 pence to £10.000. Wheel B always had a 2.5 times higher amount

than wheel A but changing probabilities for each amount size, ranging from ~5% (1/18) to 100%. An indifference point was determined for each reward size whenever participants switched from wheel A to wheel B or vice versa after performing the procedure twice. Their main finding was that the indifferent points were not significantly different between the ATD and placebo group and therefore no difference in the willingness to take risks. Two studies that used the Cambridge gambling task introduced by Rogers et al. (1999) reported opposite effects of ATD on probability weighting of gains. In this task, participants had to guess whether a token is hidden in one of the red or one of the blue boxes presented on a screen. There were always 10 boxes shown, and the ratio of red and blue boxes varied from e.g. 4:6 to 9:1. Once they made their choice, they also bet a certain percentage of points on their choice. The goal was to accumulate as many points as possible by guessing the correct colour and betting already gathered points. It should be noted that, in contrast to our probabilistic choice tasks, there was no sure option. Rogers et al. (1999) found that participants in the ATD group more often chose the box colour with fewer number of boxes compared to the other colour and therefore had a less likely positive outcome, compared to the placebo group. However, as stated above, Talbot et al. (2006) used the same task and found the opposite result, namely that participants in the ATD group chose the colour with a higher number of boxes (i.e. more likely positive outcome) more often, compared to the placebo group.

The reason for these opposing findings is yet unclear as the study designs, the amino acid mixture and demographics were very comparable. Interestingly, a recently published article suggests that the net effect of ATD on risky decision-making is depending on peripheral baseline levels of 5-HT1B receptor mRNA (Faulkner et al., 2017), highlighting the importance to consider individual differences that might modulate the effects of the tryptophan interventions. In more detail, they used ATD and a sham condition to investigate changes in tonic 5-HT levels in a gambling task developed by Rogers et al. (2003). This task included both gains and losses, gains-only and losses-only trials. The gain/losses-only trials offered a certain win/loss of 40 points or a 50% chance of winning/losing 80 points. Trials that involved both outcomes consisted of a control gamble where always 10 points could be won or lost with a 50% chance and an experimental gamble with a high (75%) or low (25%) probability to win either a small (20) or large (80) or lose a small (20) or large (80) amount of points. Faulkner and colleagues did not observe a main effect of intervention, but when they investigated the contrast ATD minus sham and controlled for 5-HT1B receptor mRNA levels, they found that individuals with higher 5-HT1B receptor mRNA levels chose the experimental gamble more

often and were more sensitive to wins (i.e. large wins). The same group (Faulkner, Selvaraj, Pine, Howes, & Roiser, 2014) provided more evidence for the importance to target specific receptor types in a PET study, where they used the same task and found a positive relationship between the availability of 5-HT1A receptors in the hippocampus and choices for the experimental gamble when the probability of winning was high. These individual differences may have accounted for discrepant findings between Rogers et al. (1999) and Talbot et al. (2006). Therefore, measuring biomarkers such as 5-HT1B receptor mRNA levels may be fruitful avenue for future tryptophan intervention studies to evaluate treatment effects on behaviour.

Finally, Rogers et al. (2003), who developed the task described in the previous paragraph, observed that the ATD group, compared to placebo, chose the experimental gamble less often when the expected amount of points was large, but more often when the expected amount was small. They did not observe an effect on losses, nor probabilities. Furthermore, of interest for our study, they did not find an effect in the gains/losses-only trials.

Taken together, previous work does not strongly support a net effect of tonic 5-HT on probabilistic decision-making. This is also reflected in our data, as we did not observe any significant effect in our tasks and supports a recent review that concludes that there is no major role of 5-HT in probabilistic discounting (Faulkner & Deakin, 2014). One may argue that previous studies were not able to reliably detect an effect of tryptophan depletion, because the sample sizes were too small and the between-subject study designs were not robust enough, resulting in insufficient power.

In contrast, our study investigated a much larger sample (n = 105), applied tryptophan depletion and loading in one within-subject study, and comprehensively assessed probabilistic decision-making with three different tasks. A sensitivity analysis with G*Power revealed that we were able to detect small to medium differences for both the main effect of intervention (f=.11) and interaction with genotype (f=.12) given a power of 80% and an error rate of 5% which should be sufficient for our purposes (Faul, Erdfelder, Lang, & Buchner, 2007). Still, we did not observe significant changes in decision-making behaviour in any of the tasks.

Another possible reason for a type II error might be that the tryptophan interventions did not work as expected. However, the Tryptophan/∑LNAA ratio change between baseline and ~3 h after application (when assessments started) showed a reduction of 70% during ATD and an increase of 122% during ATL. This indicates that the intervention worked as expected. It should

be noted that, as part of the limitations of this study described later in the discussion, the effects of acute tryptophan interventions are not completely understood and therefore the extent to which these interventions affect 5-HT levels is still under debate (Crockett et al., 2012; van der Plasse, 2013). Thus, we cannot completely exclude the possibility that our interventions did not substantially alter neural 5-HT levels. Additionally, we cannot exclude the possibility that inter-individual differences related to the 5-HT system such as 5-HT1B availability (Faulkner et al., 2017) may have masked the effects of the tryptophan intervention.

Furthermore, it has been previously reported that genotype may moderate the effect of the intervention (Blair et al., 2008; Finger et al., 2007; Marsh et al., 2006; Roiser et al., 2006), i.e. having a stronger effect on L/L individuals. To tackle this issue, we stratified our sample for genotype and did not observe a significant interaction between intervention and genotype, which excludes this possibility.

It might also be possible that the tasks of the VBDM were not sensitive enough to measure condition related changes in choice behaviour. Although this is the first study to use the battery in the context of a tryptophan intervention, we have evidence that the battery is able to detect group differences between healthy controls and detoxified patients with alcohol use disorder (Bernhardt et al., 2017). Therefore, in our opinion, the battery should also be able to detect within-group differences in this study setting. Moreover, despite the intervention, the within-task correlations in PDG and MGA remained moderate positive across sessions and varied from small to moderate positive in PDL (supplemental material, Tables S1/S2/S3). Considering these points, it is unlikely that a potential true effect remains undetected with our study design and sample size.

2.4.2. Genetic study

2.4.2.1. Higher risk seeking for losses in S-allele carriers

Although no effect of intervention could be detected, we found that *5-HTTLPR* was associated with risk-seeking for losses as measured with the PDL task. Notably, genetic variations in *5-HTTLPR* have been previously found to contribute to probabilistic choice. Similar to the results of previous studies that used probabilistic outcomes and framed the expected outcomes as gains (i.e. keep 20 of 50 Euro) or losses (i.e. lose 30 of 50 Euro) (Crisan et al., 2009; Roiser et al., 2009), we found that individuals with the S/S genotype showed increased risk-seeking for losses, but not for gains, compared with the other genotype groups. However, this finding supports the

idea that S-allele carriers might be more susceptible to losses, possibly because they are more arousing and may trigger increased physiological responses, especially in S-carriers. Crisan et al. (2009) provided some evidence for this loss related physiological response as they measured increased skin conductance responses in S-allele carriers when confronted with sure or probabilistic losses compared to the L/L genotype group. Increased activity of the amygdala in S-allele carriers is suspected to mediate higher arousal levels when outcomes are framed as losses in their probabilistic choice task compared to the L/L genotype (Roiser et al., 2009). The hyperactivity of the amygdala in S-allele is believed to be the result of a structural rewiring in corticolimbic areas of the brain, influenced by *5-HTTLPR* in early developmental stages. In this regard, rat and mouse models have shown that the absence of SERT and excessive 5-HT levels in the synaptic cleft leads to lasting structural changes in the central nervous system (Holmes, Lit, Murphy, Gold, & Crawley, 2003; Kim et al., 2005) and may lead to increased anxiety in adulthood (Ansorge, Zhou, Lira, Hen, & Gingrich, 2004).

This line of evidence suggests a dissociable mechanism for the effect of the 5-HT system on decision-making, which is not related to acute changes in tonic levels but rather to genetic variations that shape structural aspects of the 5-HT system. This is insofar interesting, as we reasoned that the S-allele is associated with higher tonic 5-HT levels, given the reduced availability of 5-HTT. This implies that the S/S genotype group may react strongest to ATD (and the L/L genotype group strongest to ATL). On the contrary, we did not observe any interaction effect in our tasks; hence, differences in value-based decision-making behaviour may be related to structural changes in neuronal plasticity. fMRI studies in humans have revealed morphological and functional changes in the corticolimbic system, with S-allele carriers showing increased activity for aversive pictures and reduced grey matter in the amygdala, as well as in the perigenual anterior cingulate cortex and reduced functional connectivity between these regions (Heinz et al., 2005; Kobiella et al., 2011; Pezawas et al., 2005). In contrast to morphological changes in grey matter, less is known about the influence of *5-HTTLPR* on white matter microstructure. Only one study investigated the uncinate fasciculus using diffusion tensor imaging, a tract that connects regions of the temporal lobe and the limbic system with the prefrontal lobe. They found reduced fractional anisotropy in the left-hemispheric uncinate fasciculus, indicating reduced structural connectivity (Pacheco et al., 2009). The question, how *5-HTTLPR* related differences in structural connectivity influence probabilistic decision-making, has not been addressed so far.

2.4.2.2. No association between genotype and risk seeking for gains and loss aversion

It should be noted that we did not observe increased risk aversion for gains in S-allele carriers nor an association between *5-HTTLPR* and loss aversion as reported previously (Ernst et al., 2014; He et al., 2010; Kuhnen & Chiao, 2009). However, the findings reported by Kuhnen and colleagues were not consistently observed in other studies that also found no relationship between risky choice for gains and *5-HTTLPR* (Anderson, Dreber, & Vestman, 2015; Juhasz et al., 2010; Lage et al., 2011; Zhong et al., 2009). Nevertheless, we cannot exclude that methodological differences may account for the different findings because Kuhnen and colleagues (Kuhnen & Chiao, 2009; Kuhnen et al., 2013) used an investment task to assess risk aversion while our task measured choice preference for sure and probabilistic gains. Interestingly, Kuhnen and Chiao (2009) also investigated a dopamine receptor polymorphism (DRD4) which they associated with reduced risk-aversion for gains. It would be tempting to test whether DRD4 is also related to reduced risk-aversion for gains in our data, which would underline the role for dopamine in reward and for serotonin in punishment processing.

With respect to loss-aversion as measured with the MGA task, we did not replicate the finding from He et al. (2010) who found increased loss aversion for homozygous S-allele compared to L-allele carriers although we used a very similar task and had a similar sample size (n = 577 vs. 517). One possible reason is that they investigated only young college students (mean age 20 years) while our sample consisted of a community sample that was 12 years older on average. Another reason may be related to task length. We estimated loss-aversion within 40 trials, while He and colleagues used data from 256 trials which provides more power to detect differences between genotypes. The study from Ernst et al. (2014) had a much smaller sample size (n = 66) with 39 healthy and 27 anxious adolescents. They used the tri-allelic *5-HTTLPR* model and found the opposite behaviour in the anxious group, i.e. adolescents with L_A/L_A showed the highest loss-aversion. Due to the strong heterogeneity of results, replication studies are highly necessary to shed more light on the potential involvement of *5-HTTLPR* in loss-aversion.

2.4.3. Limitations

A few limitations of the study should be noted. First, it is assumed that the intervention has a rather modest effect on the 5-HT system compared to drugs such as SSRIs (Cools, Roberts, & Robbins, 2008). There are also possible interactions with other neurotransmitter systems, which may reduce the sensitivity to an extent that our tasks could not measure potential differences

anymore despite of our strong design and sample size (van der Plasse, 2013; van Donkelaar et al., 2011). A strong candidate is dopamine, which has been found to interact strongly with 5-HT in brain regions, that are important for decision-making (Esposito, Di Matteo, & Di Giovanni, 2008; Fischer & Ullsperger, 2017). Therefore, future studies should take into account also markers of dopamine transmission efficiency (i.e. catechol-O-methyl transferase, COMT; cf. Ramsøy & Skov, 2010).

2.4.4. Conclusions

Overall, we tested the effects of 5-HT in the context of the framework by Cools et al. (2011), which assumes that tonic 5-HT changes the overall average outcome rate, which allows to explain different effects of 5-HT on value-based decisions. We found that neither ATD nor ATL influenced decision-making behaviour. This is in contrast to the assumed role of 5-HT suggested in the framework and therefore does not support a key role of tonic 5-HT levels for value-based decision-making as measured with our VBDM battery. However, we found a significant association of *5-HTTLPR* and risk-seeking for losses, that is, S/S individuals were more risk-seeking compared to S/L and L/L individuals. On the other hand, we did not observe changes in risky choice behaviour after depleting or increasing 5-HT, which makes a direct connection between *5-HTTLPR* and 5-HT levels unlikely. Furthermore, the genotype association did not extent to the gain domain, which emphasizes the robust influence of the 5-HT system in aversive processes such as losses or punishments (Crisan et al., 2009; Crockett & Cools, 2015; den Ouden et al., 2013; Finger et al., 2007). Finally, based on our data we conclude that the 5-HT system is specifically involved in the processing of losses, independent of temporary changes in tonic 5-HT levels, presumably associated with morphogenetic changes in neurotransmission mediated by *5-HTTLPR* during brain development.

3. Study 2 – No Evidence for the Involvement of Serotonin or the *5-HTTLPR* Genotype in Intertemporal Choice in a Larger Community Sample

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Abstract

Background: Serotonin (5-HT) has been implicated in impulsive behaviours such as temporal discounting. While animal studies and theoretical approaches suggest that reduced tonic 5-HT levels increase temporal discounting rates and vice versa, evidence from human studies is scarce and inconclusive. Furthermore, an important modulator of 5-HT signalling, a genetic variation in the promoter region of the serotonin transporter gene (*5-HTTLPR*), has not been investigated for temporal discounting so far.

Objective: First, to test for a significant association between *5-HTTLPR* and temporal discounting. Second, to investigate the effect of high/low tonic 5-HT levels on intertemporal choice and BOLD response, controlling for *5-HTTLPR.*

Methods: We tested the association of *5-HTTLPR* with temporal discounting rates using an intertemporal choice (ITeCh) task in 611 individuals. We then manipulated tonic 5-HT levels with acute tryptophan interventions (depletion, loading, balanced) in a subsample of 45 S-allele and 45 L/L-allele carriers in a randomized double-blind crossover design using fMRI and an ITeCh task.

Results: Overall, we did not find any effect of 5-HT and *5-HTTLPR* on temporal discounting rates or the brain networks associated with valuation and cognitive control.

Conclusion: Our findings indicate that 5-HT may not be directly involved in choices including delays on longer timescales such as days, weeks, or months. We speculate that 5-HT plays a stronger role in dynamic ITeCh tasks where the delays are on a timescale of seconds and hence are therefore directly experienced during the experiment.

3.1. Introduction

Mundane decision making often involves decisions with an immediate outcome, such as, choosing between two dishes. Another typical situation is to choose whether to spend money now or to invest it for later benefits. These kinds of choices, when individuals weigh outcomes associated with variable delays until harvesting a benefit, have been termed intertemporal choices (ITeCh) and they are an integral part of our daily life.

To get an estimation on how much impact a delay has on ITeCh in an experimental context, researchers usually present participants with a choice between a smaller but sooner/immediate amount of money and a larger but later amount of money. How strongly an amount is devalued (discounted) as a function of the delay, can be well described by a hyperbolic discounting function with a free parameter k, a larger value indicating stronger discounting behaviour (Bickel, Odum, & Madden, 1999; Mazur, 1987).

Specific evidence for the involvement of 5-HT neurons in waiting for delayed rewards comes from recent experiments in rats. Single-unit recordings from 5-HT neurons in the dorsal raphe nucleus showed increased firing rates when rats successfully waited for food rewards, and reduced firing rates predicted failures to wait for potential future rewards (K. Miyazaki, K. W. Miyazaki, & Doya, 2011). Another study that used rats and an optogenetic approach to increase 5-HT neurotransmission in the dorsal raphe nuclei also found that 5-HT activity promoted waiting for delayed rewards (Fonseca, Murakami, & Mainen, 2015).

Newer computational approaches suggest that the discounting behaviour in ITeCh is governed by the opportunity costs of time, meaning that the value of a delayed offer is reduced by the cost associated with the waiting time until reception (Boureau & Dayan, 2011; Cools et al., 2011). Together, the neuromodulators 5-HT and dopamine (DA) are important in signalling said costs in a way that low 5-HT (or high DA) levels in the brain reflect increased opportunity costs and thus waiting becomes more costly, promoting choices for a smaller but immediate reward (Cools et al., 2011). Vice versa, high 5-HT (or low DA) levels reduce opportunity costs and therefore promote waiting for a larger reward.

This intriguing model received some support from two human studies: one used L-Dopa, the other an acute tryptophan (a precursor of serotonin) intervention to manipulate DA and 5-HT levels in the brain, respectively. In agreement with the framework, an increase of DA via L-Dopa led to increased temporal discounting rates compared to placebo (Pine, Shiner, Seymour,

& Dolan, 2010). On a neural level, this was reflected by a reduced BOLD response in brain regions associated with the encoding of subjective value of a delayed offer, i.e. striatum and orbitofrontal cortices (Pine et al., 2010). The other study manipulated 5-HT levels using an acute tryptophan intervention with a depletion and a loading condition. They reported that the depletion condition increased temporal discounting compared to the loading and sham condition (Schweighofer et al., 2008), but the loading condition had no effect compared to the sham condition. In an earlier fMRI study, the same group reported that high and low 5-HT modulated different loci of the striatum while processing subjective values, highlighting its role in the evaluation of immediate and delayed rewards (Tanaka et al., 2007). Nevertheless, it should be noted that not all studies, which used a tryptophan intervention, found significant effects (Crean, Richards, & de Wit, 2002; Demoto et al., 2012; Worbe, Savulich, Voon, Fernandez-Egea, & Robbins, 2014).

On the neural level, there is emerging consensus that brain regions activate to distinct facets of the ITeCh process, such as valuation, cognitive control and prospection (Peters & Büchel, 2011), that integrate information about offer value and delay resulting in a choice for either the immediate or delayed amount. In this regard, a repetitive transcranial magnetic stimulation study from Figner et al. (2010), which used a single 15-minutes train of 1 Hz low-frequency pulses, provided evidence that temporary disruption of the dorsolateral prefrontal cortex (dlPFC) increased choices of the immediate amount, while the reward valuation process was not affected. In agreement with this finding, a dynamic causal modelling study by Hare, Hakimi, and Rangel (2014) suggests that the dlPFC increases connectivity to the ventromedial prefrontal cortex (vmPFC) when participants chose the delayed offer. Finally, a study that orthogonalized amount and delay information found that the dlPFC and parts of the posterior parietal cortex (PPC), including the temporoparietal junction (TPJ) are processing delay information (Ballard & Knutson, 2009). However, little is known about how acute changes in 5-HT levels influence these brain processes in humans. Therefore, one objective of our work is to test whether increased 5-HT reduces sensitivity towards delay (i.e. facilitating cognitive control) or rather increases signalling for amount magnitude, using a similar paradigm as Ballard and Knutson (2009).

Another important method to investigate the 5-HT system is to exploit naturally occurring variations in the serotonin transporter-linked polymorphic region (*5-HTTLPR*), a promoter region of the SLC6A4 gene, which codes for the 5-HT transporter (5-HTT), a protein that is important for extracellular 5-HT regulation. The short (S-) allele has been associated with

reduced transcriptional activity and mRNA expression compared to the long (L-) allele in vitro. Association studies suggest an involvement of *5-HTTLPR* genotype in a variety of personality and behavioural phenotypes (Homberg & Lesch, 2011; Lesch et al., 1996). Previous studies suggest that S-allele carriers have a more cautious decision-making style when performing tasks involving risky choices (Crisan et al., 2009; Kuhnen & Chiao, 2009; Neukam et al., 2018). However, there are to date no studies about how *5-HTTLPR* is associated with ITeCh in human beings. Data from a study with macaques demonstrated less discounting for delayed offers in S-allele carriers compared to the L/L genotype (Jedema et al., 2010). Their finding is in line with the model as the reduced availability of 5-HTT in S-allele carrier has been associated with increased synaptic 5-HT levels (Heils et al., 1996; Mathews et al., 2004), although the exact mechanism is not yet fully understood. Hence, we hypothesized that S-allele carrier show reduced discounting rates compared to L/L-allele carrier. To test this hypothesis, we genotyped a large community sample (N=611) with respect to *5-HTTLPR* and estimated individual temporal discounting rates. Furthermore, to test whether temporal discounting rates are increased by low tonic 5-HT levels compared to high levels, we manipulated 5-HT in a subsample (N=112) using an acute tryptophan intervention in a double-blind crossover design with depletion (ATD), loading (ATL), and a control condition (BAL). On the brain level, we expected lower 5-HT levels to be associated with a weaker positive BOLD response for amount magnitude of the delayed offer in vmPFC/OFC and striatum but a stronger negative BOLD response in the dlPFC and parietal cortex for higher delays.

3.2. Materials and Methods

3.2.1. Genetic study

3.2.1.1. Participants

All participants were recruited from the Dresden area via mail using randomly drawn addresses provided by the local residential registry. They were screened for any physical condition that requires medical treatment, contraindication to MRI and pregnancy. Additional screening for psychiatric disorders (ICD-10) included a lifetime history of organic psychiatric disorders, opiate, cocaine, stimulants, hallucinogens, inhalants or poly substance dependence, schizophrenia or other personality/affective disorders as well as other psychiatric disorders that required medical treatment within the last year. Any positive screening outcome led to exclusion from the study. Another exclusion criterion was visual impairment (visual acuity <

0.8 with correction). A total of 611 of 1382 individuals passed the screening and gave written informed consent. The full recruitment procedure is described in Neukam et al. (2018). The study was approved by the ethic board of the Technische Universität Dresden (EK 42022012) and is in agreement with the Declaration of Helsinki.

3.2.1.2. Procedure

Participants were scheduled based on their day and time preference. After an initial introduction to the planned procedure, they first gave a blood sample to be genotyped for *5-HTTLPR* and were then guided to a muted booth equipped with a computer to fill in several questionnaires and completed an adaptive delay discounting task (DD) to estimate the temporal discounting rate. The task is described in the section Behavioural analysis (DD) and was part of a value-based decision-making battery not reported here (Pooseh et al., 2018). Participants were told that they would be paid according to one randomly selected choice out of the thirty choices they made in the DD task; in reality, immediate amounts were favoured for obvious practical reasons.

Genotyping. Blood samples were genotyped for *5-HTTLPR* at the Central Institute of Mental Health in Mannheim, Germany. The genotyping procedure is described in detail elsewhere (Dukal et al., 2015). Blood from 602 of 611 participants was available for genotyping, which resulted in the following group sizes: 99 S/S, 283 S/L and 220 L/L. The allele and genotype frequencies did not deviate from the Hardy-Weinberg equilibrium ($\chi^2(1) = 0.\ 2461$, $p = .62$). The subsample that participated in the pharmacological study and was eligible for the analysis consisted of 41 S/S, 4 S/L and 45 L/L carriers.

3.2.1.3. Delay discounting task (DD)

Individual temporal discounting rates (k) were estimated with an adaptive DD task using Bayes' rule (Pooseh et al., 2018). Participants made in total 30 choices between two simultaneously presented offers, which were displayed next to each other on the screen. One smaller offer was always available immediately; the other larger offer was available after a certain delay. A chosen offer was highlighted with a red frame until the next trial started. The position (left or right) of immediate and delayed offers on the screen was random. The amount range of the task was 5-30 Euro and the delays were three days, one week, two weeks, one month, two months, six months and one year. There was no time limit for each decision and the mean decision time was (3.8 ± 1.41 seconds). We estimated discounting behaviour using the well-known hyperbolic function $SV = A/(1 + k \times D)$, where A is the offered amount magnitude, D is the delay in days

after which A is available, and SV is the subjective value of A after taking D into account (Mazur, 1987). Note that when D = 0, SV = A. The likelihood of choosing between the two offers follows a softmax probability function, in which $\beta > 0$ serves as a consistency parameter such that larger values correspond to a higher probability of taking the more valuable option. The equation for this function is $P(a_i|k,\beta) = \exp(\beta Q(a_i))/(\exp(\beta Q(a_i)) + \exp(\beta Q(a_d))$, which is the probability of choosing the immediate amount a_i given k and β. The probability of choosing the delayed amount a_d is consequently $P(a_d|k,\beta) = 1 - P(a_i|k,\beta)$, where $Q(a_i)$ and $Q(a_d)$ are the action values of taking the immediate and delayed amount respectively. After observing a choice at each trial, the belief about the parameters is updated using the Bayes' rule $P(k,\beta|choice) \propto P(choice|\, k,\beta)P(k,\beta)$. We assume normally distributed starting priors with mean values of ln(k) =-2.5 and ln(β) = 0, which are in the middle of the allowed parameter range ($-15 \leq \ln(k) \leq 10$, $-8 \leq \ln(\beta) \leq 8$, and large variances ($\sigma^2 = 30$) to avoid strong initial biases (Pooseh et al., 2018). The task was implemented in MATLAB (R2010a, The MathWorks, Inc., Natick, Massachusetts, United States) using Psychtoolbox 3.0.10 based on the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997).

3.2.1.4. Behavioural analysis (DD)

The model parameters were analysed using SPSS 23 (IBM, Chicago IL, United States). The *k*-values were in logarithmic scale resulting in approximately normally distributed data. They were then used as a dependent variable in a univariate analysis of variance (ANOVA) with the two factors genotype and sex. Significance was assumed at $p < .05$. We furthermore computed a Bayesian ANOVA using JASP (JASP Team (2019), Version 0.10) with the same factors to estimate the Bayes Factor (BF) comparing the marginal likelihoods between the null and alternative hypothesis. This analysis contributes to the frequentist ANOVA by allowing statements about the actual presence of an effect instead of simply reject or fail to reject the null hypothesis. We considered BFs smaller 1/100 to be extreme evidence for H0, BFs between 1/100 and 1/30 very strong evidence for H0, BFs between 1/30 and 1/10 strong evidence for H0, BFs between 1/10 and 1/3 moderate evidence for H0, BFs between 1/3 and 1 anecdotal evidence for H0, a BF of 1 no evidence, BFs between 1-3 anecdotal evidence for H1 and BFs > 3 moderate evidence for H1 (Jeffreys, 1961).

3.2.2. Pharmacological study

3.2.2.1. Procedure

The experiment and results described in this manuscript are part of a larger project that investigates the influence of 5-HT on several decision-making parameters and fMRI measures (Deza-Araujo et al., 2019; Neukam et al., 2018). For this study, we aimed for 100 homozygous subjects. Participants were re-invited for this randomized, double-blind, cross-over study based on the inclusion criteria described in the section Procedure above. In the first session, a urine test was conducted to test for illicit drug use (Kombi/DOA10-Schnelltest, MAHSAN Diagnostika GmbH, Reinbek, Germany) and in every session breath-alcohol was measured (Alcotest 6510, Dräger, Lübeck, Germany). The tryptophan interventions were scheduled on three separate dates with an interval of at least one week. They were told to abstain from protein-rich food and alcohol and consume meals with a large proportion of carbohydrates the day before and to fast 12 hours before their appointment. Participants arrived either 08:30 a.m. or 10:30 a.m. at the Neuroimaging Center (NIC), signed the informed consent form and received a brief introduction before the amino acid drink, consisting of the amino acid powder dissolved in 300-500 ml water and carbon dioxide-free Sprite©. The order of the mixtures applied in each session was randomized and both the experimenter and the participant were unaware of the condition. Sugar-free candies and water were provided ad libitum to minimize side effects. After they ingested the mixture, participants filled in several questionnaires and performed computerized tasks related to cognitive and emotional states. MRI scanning started approximately 3 hours after ingestion of the mixture. After around 6.5 hours, the session was completed and participants were compensated 60 Euro plus their earnings from the tasks (for more details on the procedure and behavioural assessment see Neukam et al., 2018).

3.2.2.2. Amino Acid Mixture

Central tryptophan levels were manipulated with a composition based on the 'Moja-De' protocol (Dingerkus et al., 2012). In this protocol, the amount of amino acids is adapted to body weight and is less than what is used in other studies (Young et al., 1985). The main advantage of this mixture is that it reduces the risk of nausea that has been reported earlier (Booij, van der Does, Haffmans, Spinhoven, & McNally, 2005; Cools et al., 2005; Schmitt et al., 2000) while still effectively changing 5-HT levels (Biskup et al., 2012; Dingerkus et al., 2012). The dose of large neutral amino acids (LNAA) was the same for all conditions and consisted of L-

phenylalanine (132 mg/kg), L-leucine (132 mg/kg), L-isoleucine (84 mg/kg), L-methionine (50 mg/kg), L-valine (96 mg/kg), L-threonine (60 mg/kg) and L-lysine (96 mg/kg). In the depletion condition (ATD) no tryptophan was added, in the balanced condition (BAL) 7 mg/kg was added and, in the tryptophan loading condition (ATL), 70 mg/kg. The amino acid compositions were prepared by Amino-Factory, Lindenberg, Germany.

3.2.2.3. Biochemical measures

A peripheral venous catheter (Braunüle®) was inserted into a vein in the bend of an elbow or, if proper insertion was not possible, in a vein on the back of a hand to collect four blood samples throughout each session: one baseline sample before the ingestion of the mixture (T0), one an hour after drinking the mixture (T1), one ~3 hours after drinking the mixture (T2), and the last one shortly before the end of the session (~6 hours after drinking the mixture, T3). Blood samples were collected in EDTA tubes (Sarstedt, Germany) and immediately centrifuged for 10 minutes at 4000g, 4°C. Plasma was then stored at -81°C until analysis. The amino acid analysis was performed as described in Henle et al. (1991). In brief, the separation was performed on a PEEK column filled with the cation exchange resin LCA K07/Li (150mm×4.6 mm, 7µm) using the amino acid analyser S 433 (Sykam, Fuërstenfeldbruck, Germany) with a gradient program utilized previously (Hellwig et al., 2011). The buffers for this lithium system were purchased from Sykam. To detect the amino acids, postcolumn derivatization with ninhydrin was applied and the absorbance of the effluent was recorded with a two-channel photometer simultaneously working at 440 and 570 nm, respectively. An external calibration was performed using a commercial amino acid mixture obtained from Sigma-Aldrich (Steinheim, Germany).

3.2.2.4. fMRI ITeCh task

We designed our ITeCh task similar to the one published by Ballard and Knutson (2009), which has the advantage that amount and delay information can be orthogonalized to specifically test the influence of 5-HT on delay-related activation. The main difference of our task is that we tailored our offers around the individual indifference point to provide the same number of attractive and unattractive delayed offers to all participants based on their k values, which were estimated by the DD task in the first part of the study. In order to achieve this aim, we first computed the individual immediate amount (IM) which was equal to the subjective value of 30 Euro after 30 days (IM: SV = 30 Euro / (1 + k × 30 days)). Next, we applied the following

formula to compute nine different amounts to an accuracy of 1 cent: Amounts = IM + c × (30 Euro – IM), where the parameter c was set to 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0 and 6.0. The delays were seven days, fourteen days, twenty-one days, thirty days, two months, three months, four months, and six months. Additionally, we had nine catch trials with a zero delay. Combining amounts and delays resulted in four offers that had a smaller and four that had a larger subjective value than the immediate amount and one offer that was at the indifference point (in total 81 trials). To avoid showing the same nine amounts for each of the nine delays, we added noise from a normal distribution with a mean of zero and a standard deviation of one Euro. An example is shown in the supplemental material Table S4. The 81 delayed offers were presented in a random order. In the introduction of the task, participants were informed of their IM, which remained the same during the task across all sessions and was only shown whenever it was chosen. A trial example is shown in Fig. 3. The presentation location of the exclamation mark on the screen was counterbalanced throughout the experiment to avoid lateralization effects of response. The task duration for all 81 trials was 25.5 minutes. As for the DD task, participants were told that one of their choices would be randomly picked and paid either immediately after the session in cash or via bank transfer according to the delay in order to increase ecological validity.

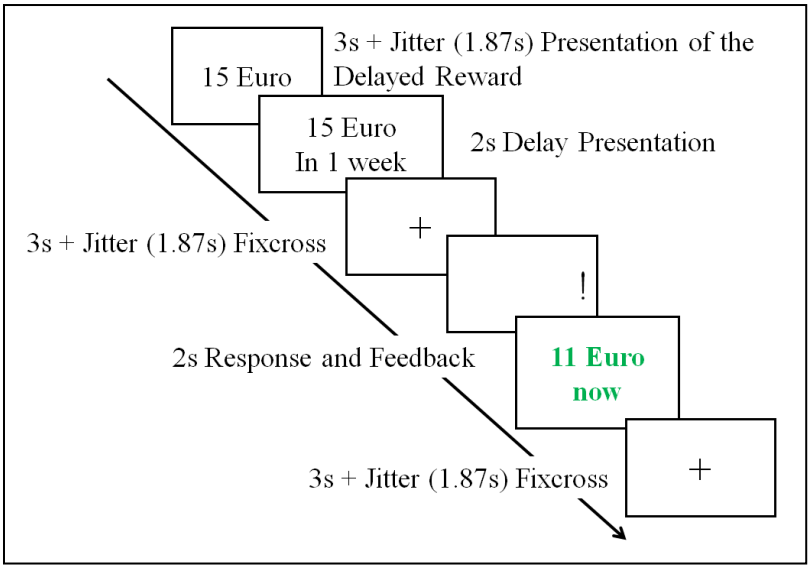

Fig. 3 Example trial of the ITeCh task used during fMRI. Participants learned before task begin the immediate amount, against which to compare the delayed offers. The jitter was taken from a truncated geometric function, ranging from 0 seconds to 9.64 seconds with a mean duration of 1.87 seconds.

3.2.2.5. MRI data acquisition

MRI images were acquired on a 3 Tesla whole-body Magnetom Trio Tim scanner (Siemens Healthcare GmbH, Erlangen, Germany), equipped with a 32-channel head coil. Functional images were acquired with an echo planar imaging (EPI) sequence (repetition time TR = 2410 ms; echo time TE = 25 ms; flip angle: 81°). Forty-two axial slices, descending slice order, with a thickness of 2 mm, aligned along the anterior/posterior commissure, were obtained with a field of view of 192 × 192 mm, an in-plane matrix size of 64 × 64 pixels, resulting in a voxel size of 3 × 3 × 3 mm (1 mm slice gap). A corresponding field map was also recorded for distortion correction of the EPI images. Structural images were acquired using a T1-weighted magnetization prepared rapid acquisition gradient echo (MP-RAGE) sequence for normalization, anatomical localization as well as screening for structural abnormalities by a neuroradiologist (TR: 1900 ms; TE: 2.26 ms; flip angle: 9°; field of view: 256 × 256 mm; 176 sagittal slices; voxel size: 1 × 1 × 1 mm). Stimuli were presented on an MR compatible screen and viewed through a rear-view mirror system. Participants responded by pressing their index fingers on two separate response devices held in each hand (ResponseGrip, NordicNeuroLab, Bergen, Norway). Presentation® Software (version 14.1, Neurobehavioral Systems, Inc., Albany, CA) was used to present the stimuli and collect behavioural responses.

3.2.2.6. Behavioural analysis (ITeCh)

Individual discounting rates were estimated from the choices made during the fMRI session, using the same algorithm as for the DD task. To improve the estimation, the k values from the DD task were used as a prior for this estimation procedure. While this part of the analysis was done with MATLAB (R2016b), the statistical analysis was done with SPSS 23. Before the analysis, *k*-values were log-transformed to obtain a near-normal distribution. Next, we tested the effect of the tryptophan intervention on discounting behaviour using a 3 (intervention) × 2 (genotype) × 2 (sex) repeated measures analysis of variance (rm-ANOVA) with intervention as within-subject factor and genotype and sex as between-subject factors. In a second 3 (intervention) × 6 (order) rm-ANOVA with discounting rate as the independent variable, we tested for potential sequence effects of intervention order by looking at the interaction with intervention. Significance was assumed at $p < 0.05$. Again, we conducted Bayesian statistics (JASP Team (2019), Version 0.10) to compute a Bayesian rm-ANOVA with the factors intervention, genotype and sex to estimate the Bayes Factors (BF) using the same interpretations as outlined above in the section Behavioural analysis (DD).

3.2.2.7. MRI data analysis

Functional and structural images were processed using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK) within the Nipype framework (Version 0.9.2, Gorgolewski et al. (2011)). All volumes were slice-time corrected (reference: slice 21), realigned to the first volume to correct for motion and distortion-corrected based on the field map acquired immediately before the scan. Next, the T1 image was co-registered to the mean EPI image and normalization to MNI space using the segment tool. The resulting transformation parameters were then applied to the distortion-corrected EPI images (resampled to 2 x 2 x 2 mm^3). Finally, normalized EPI images were spatially smoothed with an isotropic Gaussian kernel (full width at half maximum = 8 mm).

First-level statistics included high-pass filtering at 128 s and a general linear model (GLM) to set up the following event-related design, using delta functions and the canonical hemodynamic response function (HRF) for convolution of all onset regressors. Included were a pair of regressors for parametrically modulated amount at the onset of the amount display and another pair for modulated delay at delay onset. For comparison reasons with Ballard and Knutson (2009), we also included for the latter pair the amount × delay interaction term as a second parametric modulator. Furthermore, we modelled two motor response regressors (left and right) and two regressors (for amount and delay) that modelled trials where no response was given. Additional, six movement regressors of no interest were included to correct for head translation and rotation (three regressors each in x, y, z directions).

To test for main effects and interactions of intervention and genotype with respect to amount and delay processing, individual parametric amount and delay contrast images were entered into a multivariate analysis of variance on second level using the recently developed "Multivariate and repeated measures" (MRM) toolbox v0.6 by McFarquhar et al. (2016). This toolbox allows voxelwise unconstrained covariance structures for the within-subject data to avoid assumptions about the form of the covariance matrix. Furthermore, MRM provides hypothesis testing using permutations (based on the *randomise* algorithm developed by Winkler, Ridgway, Webster, Smith, and Nichols (2014)) to construct an empirical distribution under the null hypothesis. We used a cluster-forming threshold of $p < .001$ (uncorrected) and 5000 permutations to generate familywise error (FWE) corrected p values $< .05$ at cluster level. In a first step, we located brain regions that are correlated with amount magnitude and delay duration by computing the respective average task contrast, which was defined as the average

across intervention and *5-HTTLPR* groups (L = [1 1], M = [1 1 1] in MRM toolbox notation). Next, we extracted parameter estimates of the BOLD signal from brain regions that showed a significant correlation with amount magnitude and delay duration, respectively to specifically test for effects of the tryptophan intervention and genotype. To this end, 8mm diameter spheres were drawn around local peak voxel activations using the MarsBaR toolbox (http://marsbar.sourceforge.net) and mean values were computed. Where applicable, the extracted data from bilateral spheres was combined because we had no a priori assumptions about lateralization. These estimates were then subjected to rm-ANOVAs (five in total) with the factors intervention and genotype. Finally, we conducted a whole-brain analysis to further explore main and interaction effects in other regions of the brain using the MRM toolbox and the respective contrasts (main effect genotype*:* L = [1 -1], M = [1 1 1]; main effect intervention: L = [1 1]; M = [1 -1 0; 0 1 -1]; interaction effect: L = [1 -1], M = [1 -1 0; 0 1 -1]).

3.3. Results

Demographic information are shown in Table 5.

Table 5 Demographic information

	N (female)	Age (SD)
Genetic study		
S/S	97 (42)	31.73 (5.9)
S/L	276 (134)	32.65 (5.5)
L/L	216 (99)	33.07 (5.6)
Statistic	$\chi^2(2) = .896$, ns.	$F(2,586) = 1.96$, ns.
Pharmacological study		
S	45 (13)	32.31 (6.1)
L/L	45 (24)	31.82 (5.9)
Statistic	$\chi^2(1) = 5.553$, $p < .05$	$F(1,88) = .15$, ns.

3.3.1. Study 1: Genetic study

3.3.1.1. Participants

Of the 602 genotyped participants, 13 had missing data, leaving 589 data sets eligible for analysis. Mean age was 32.7 ± 5.6 years. The distribution across genotype groups was as follows: S/S: 97 (42 females), S/L: 276 (134 females), L/L: 216 (99 females). Sex did not significantly differ across genotype groups ($\chi^2(1) = 0.896$, $p = .639$). Age was not significantly different across genotype groups ($F(2, 586) = 1.958$, $p = .142$).

3.3.1.2. 5-HTTLPR and delay discounting

Levene's test for equality of error variances was not significant ($F(5,583) = 0.811$, $p = .542$), indicating that variances were homogeneous across genotype and sex. A univariate analysis of variance (ANOVA) demonstrated no main effect of genotype groups ($F(2, 583)= 0.668$, $p = .513$, $\eta^2_p = .002$) or sex ($F(1, 583) = 0.064$, $p = .801$, $\eta^2_p = .000$) on the discounting parameter

k. Furthermore, there was no significant interaction of these two factors ($F(2, 583) = 0.436$, $p = .647$, $\eta^2_p = .001$). Fig. 4(a) shows a graphical depiction of the data.

Additionally, the Bayesian ANOVA revealed strong evidence for no association of genotype ($BF_{10} = 0.041$), moderate evidence for no effect of sex ($BF_{10} = 0.105$) and extreme evidence for no interaction of genotype and sex ($BF_{10} = 0.000286$) on the discounting rates.

3.3.2. Study 2: Pharmacological study

3.3.2.1. Participants

170 participants were invited to the study. Of those, 37 dropped out due to negative side effects of the mixture and 21 declined further participation due to personal or time reasons. Of the remaining 112, who successfully completed the study, five received accidently the same amino acid mixture in all sessions and had to be excluded. Of the remaining 107 participants, data from two could not be used because the task crashed in one session for one participant and for the other a faulty set of choice pairs was created. Finally, we excluded one participant due to normalization failure and 14 participants due to excessive motion in the MR scanner (translation > 2mm volume-by-volume in any direction) and MRI artefacts (i.e. insufficient brain coverage, ghosting, distortions, spiking), leaving 90 participants eligible for the analyses. Due to a lack of participants homozygous for the S-allele, we filled the group with heterozygous participants, which are more similar to S/S than to L/L with respect to mRNA expression (Bradley, Dodelzon, Sandhu, & Philibert, 2005; Lesch et al., 1996). The genotype distribution of the sample was 41 S/S, 4 S/L (termed S-carrier group in the next sections), and 45 L/L.

An analysis of variance showed that the two genotype groups did not differ in age ($F(1, 88) = 0.148$, $p = .701$). There was however a significant difference of genotype with respect to sex ($\chi^2(1) = 5.553$, $p = .018$), indicating a lower ratio of females in the S-carrier group compared to the L/L group.

3.3.2.2. TRP/ΣLNAA blood plasma analysis

Complete data from 80 participants were available. In a first step, we used the TRP/ΣLNAA ratios, measured at four time points per session to compute the area under the curve (AUC) as a representative parameter of the tryptophan intervention. To account for individual differences in baseline TRP/ ΣLNAA levels, we subtracted the reference time point (T0) from each of the three remaining time points (T1-T3). Therefore, AUC was computed from three time points

and negative values for the AUC could occur in the data. The AUC score was entered as the dependent variable into a 3 (intervention: ATD vs. BAL vs. ATL) by 2 (genotype: S vs. L/L) by 2 (sex: male vs. female) rm-ANOVA. The analysis revealed a strong main effect of intervention ($F(1.15, 87.63) = 305.97$, $p = 5.1\times10^{-54}$, $\eta^2_p = .801$, Greenhouse-Geisser (G-G) corrected). A follow-up linear contrast analysis showed a significant difference between ATD and BAL ($F(1, 76) = 54.220$, $p = 1.8\times10^{-10}$, $\eta^2_p = .416$) as well as between BAL and ATL ($F(1, 76) = 232.844$, $p = 7.6\times10^{-25}$, $\eta^2_p = .754$). These findings clearly show that participants had the highest TRP/ ΣLNAA ratios in the ATL ($M_{AUC} = 21.05$, $SD = 10.68$) condition and the lowest ratios in the ATD ($M_{AUC} = -13.34$, $SD = 2.40$) condition (BAL, $M_{AUC} = -9.45$, $SD = 2.13$). There were no other interaction effects. There was also no main effect of genotype ($F(1, 76) = 2.864$, $p = .095$, $\eta^2_p = .036$). A graphical depiction of intervention and genotype effects on the AUC is shown in the supplemental material, Figure S3.

3.3.2.3. Temporal discounting behaviour

The discounting rates are shown in Fig. 4(b). The rm-ANOVA revealed no main effect of the intervention ($F(1.88, 161.83) = 0.231$, $p = .781$, $\eta^2_p = .003$, G-G corrected) nor interaction effects with genotype ($F(1.88, 161.83) = 0.312$, $p = .719$, $\eta^2_p = .004$, G-G corrected) or sex ($F(1.88, 161.83) = 0.316$, $p = .716$, $\eta^2_p = .004$, G-G corrected). Furthermore, there was no main effect of genotype ($F(1, 86) = 0.294$, $p = .589$, $\eta^2_p = .003$). There was however a significant main effect of sex ($F(1, 86) = 6.043$, $p = .016$, $\eta^2_p = .066$). This finding however is most likely a false positive, as we did not observe such an effect in the full sample. Finally, the second rm-ANOVA to look for sequence effects showed no significant interaction effect of intervention with intervention order ($F(9.22, 154.95) = 1.083$, $p = .378$, $\eta^2_p = .061$, G-G corrected).

Here, the Bayesian rm-ANOVA revealed strong evidence for no effect of intervention ($BF_{10} = 0.044$), extreme evidence for no interaction effect with genotype ($BF_{10} = 0.002$) and very strong evidence for no interaction effect of intervention and sex ($BF_{10} = 0.011$) on temporal discounting. Furthermore, there was anecdotal evidence for no association of genotype ($BF_{10} = 0.396$) and anecdotal evidence for an effect of sex ($BF_{10} = 2.85$).

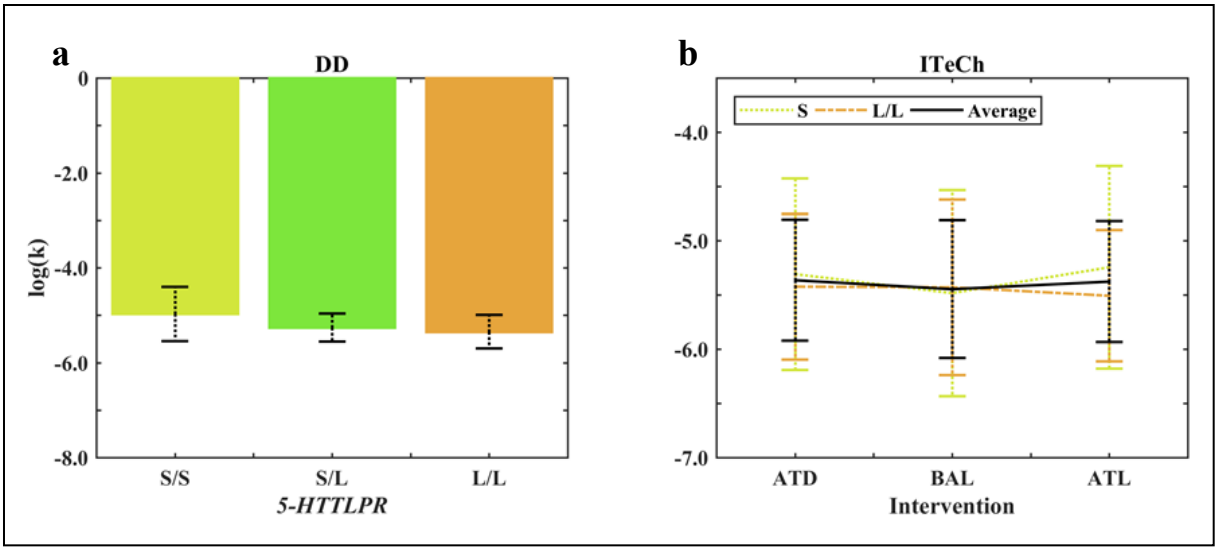

Fig. 4 Effect of *5-HTTLPR* on temporal discounting rates for (a) the genetic part of the study (DD task) as a function of *5-HTTLPR* and (b) the pharmacological part of the study with the acute tryptophan interventions ATD, acute tryptophan depletion, ATL, acute tryptophan loading, BAL, balanced. Mean scores and confidence intervals are log-scaled and bootstrapped with 10.000 iterations. The error bars indicate 95% bias-corrected and accelerated confidence intervals. No significant effects of genotype or intervention are observed.

3.3.2.4. fMRI ITeCH whole-brain analysis

A list of brain regions that showed significant correlations with amount or delay across intervention and genotype is provided in the supplemental material, Tables S5/S6. Fig. 5(a) and (b) shows the clusters and ROIs that were used for the ROI analyses.

3.3.2.5. fMRI ITeCh amount magnitude

The parametric effect of amount showed no significant cluster that survived the FWE correction; therefore, we used a more liberal threshold with an uncorrected $p < .001$ and a cluster size of at least 60 voxels. This revealed brain regions implicated in reward processing, i.e. medial frontal gyrus (peak voxel coordinates : [x = 0, y = 38, z = -10]; Wilk's $\Lambda = 16.19$; cluster size = 144) and bilateral ventral parts of the striatum (peak coordinates left: [x = -10, y = 8, z = -4]; Wilk's $\Lambda = 20.16$; cluster size = 62; peak coordinates right: [x = 10, y = 10, z = -4]; Wilk's $\Lambda = 16.19$; cluster size = 120). We did not find a significant parametric amount effect in the posterior cingulate.

3.3.2.6. fMRI ITeCh delay duration

Here, the parametric BOLD response of delay over all conditions revealed several brain areas. The largest cluster encompassed areas of the temporal, parietal and occipital lobe (i.e. BA

7/18/19/21/31/40, cluster size = 27352, p < .001 FWE corrected). The second cluster included brain regions that covered mainly parts of the superior and middle frontal gyrus as well as subcortical regions such as the thalamus and the striatum (cluster size = 8056, p < .001 FWE corrected). The third cluster consisted of several parts of the frontal cortex, mainly the inferior and middle gyrus, as well as medial parts (cluster size = 8872, p < .001 FWE corrected). The fourth cluster was located in the anterior cingulate cortex (BA 32, cluster size = 490, p < .02 FWE corrected). The fifth and sixth cluster also covered parts of the anterior cingulate as well as the middle frontal gyrus respectively (cluster size = 224, p < .05 FWE corrected; cluster size = 222, p < .05 FWE corrected).

3.3.2.7. Region of interest (ROI) analysis

For the parametric amount effect, we created two ROIs: one around peak voxels in the medial prefrontal cortex (mPFC; x = 0, y = 38, z = -10) and one for the bilateral ventral striatum (VS; x = -10, y = 8, z = -4, x = 10, y = 10, z = -4). For the parametric delay effect, we created three ROIs around peak voxels in the left dlPFC (x = -24, y = 62, z = 10), bilateral PPC (x = -28, y = -54, z = 46; x = 30, y = -56, z = 46) and bilateral TPJ (x = -54, y = -56, z = 24; x = 52, y = -48, z = 24).

Overall, the rm-ANOVAs revealed no significant main effects of intervention or genotype, nor a significant interaction effect in any of the selected brain regions associated with amount or delay processing (cf. Table 6). Additionally, the exploratory whole-brain analyses also revealed no significant effects of intervention and genotype.

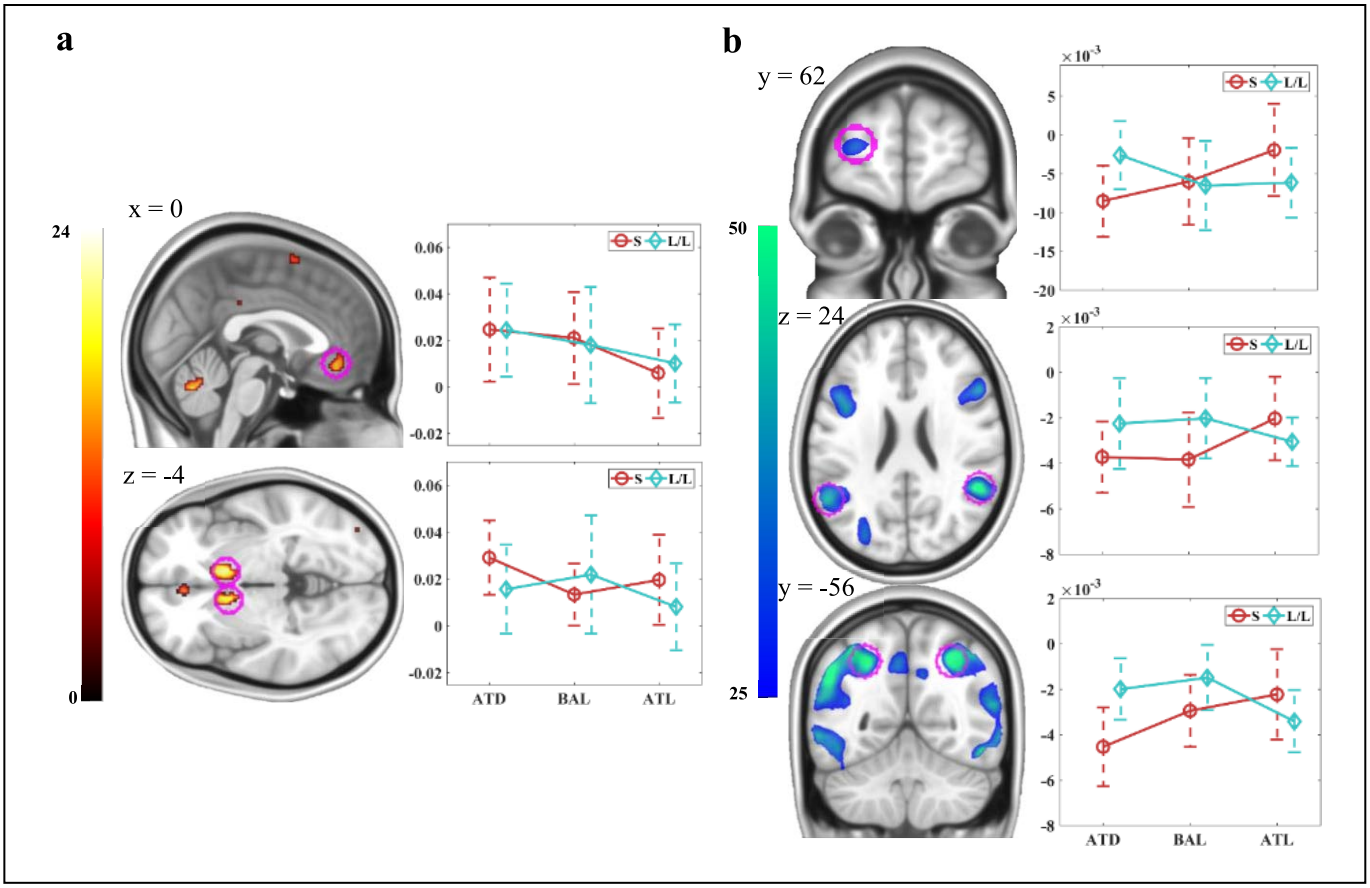

Fig. 5 fMRI results intertemporal choice task (neurological display convention, i.e. left-on-left). The activation maps depict Wilk's Λ F-scores, based on 5000 permutations, for the main effects of task. (a) Hot colours indicate significant (positive) voxel correlations with amount magnitude of the delayed offer ($p < .001$ uncorrected) and (b) cool colours indicate significant (negative) voxel correlations with delay duration of the delayed offer ($p(FWE) < .05$ cluster corrected). BOLD signal was extracted from activation loci of interest using spherical ROIs shown here in violet colour and plotted to show the contributions of the acute tryptophan intervention (depletion, ATD; loading, ATL, balanced, BAL) and of the *5-HTTLPR* genotype groups. The y-axes show the averaged BOLD magnitude across genotype group and intervention condition from all voxel values within an ROI mask. Overall, there was no significant main effect of intervention nor *5-HTTLPR* nor an interaction effect on the BOLD response. Mean scores and confidence intervals are bootstrapped with 10.000 iterations. The error bars indicate 95% bias-corrected and accelerated confidence intervals.

Table 6 Results of the repeated-measures ANOVAs from the ROI analyses

Region	Peak voxel (MNI)			F-score (p-value)		
	x	y	z	Intervention	*5-HTTLPR*	Intervention* *5-HTTLPR*
Medial prefrontal cortex	0	38	-10	1.489 (.23)	0.001 (.97)	0.068 (.93)
Ventral striatum	-10	8	-4	0.478 (.62)	0.361 (.55)	0.974 (.38)
	10	10	-4			
Dorsolateral prefrontal cortex	-24	62	48	0.357 (.70)	0.040 (.84)	1.80 (.17)
Temporoparietal junction	-54	-56	24	0.151 (.86)	0.991 (.32)	1.522 (.221)
	52	-48	24			
Posterior parietal cortex	-28	-54	46	0.875 (.42)	1.762 (.19)	2.895 (.06)
	30	-56	46			

3.4. Discussion

Our study comprehensively investigated the effects of 5-HT on intertemporal choice behaviour and its neural correlates from a genetic and pharmacological perspective. Contrary to our hypotheses, we did not observe any significant associations of *5-HTTLPR* genotype and discounting behaviour in the genetic part of the study despite our large sample size (N = 589). Furthermore, we could not detect any effects of the tryptophan interventions on discounting rates or related brain activations (N = 90). Furthermore, the BFs we computed in both studies demonstrate whether the evidence provided by the data support H0 or H1. We found in the genetic study that the H0 (genotype is not associated with discounting) is 24 times more likely than the H1 (genotype is associated with discounting), indicating strong evidence for no association. In the pharmacological study, the data suggest that it is 23 times more likely that the intervention has no effect on discounting behaviour than that the intervention has an effect, again indicating strong evidence for no effect.

In humans, there are few studies that investigated the effect of 5-HT on intertemporal choice using an acute tryptophan intervention. Three studies used (pseudo-)hypothetical offers to assess discounting behaviour. Crean et al. (2002) and Worbe et al. (2014) found no effect of

ATD on delay discounting. Although Crockett, Clark, Lieberman, Tabibnia, and Robbins (2010) reported that the extent of the pharmacokinetic effect of ATD (reduction of TRP/ΣLNAA from baseline to pretest blood levels) was correlated with increased delay discounting, they found no main effect of the intervention. Using a comparable experimental design, we also could not observe a main effect of our interventions nor an effect proportional to the pharmacokinetic parameters (supplemental material, Fig. S4). Therefore, our results might indicate that discounting behaviour during tasks that employ (hypothetical) time preferences is not substantially modulated by 5-HT neurotransmission.

On the other hand, three human studies by the same research group used dynamic intertemporal choice tasks, with delays on a shorter time scale of seconds (compared to days or months), so that participants actually experienced the delay until reward delivery when performing the task, similar to animal ITeCh tasks. Schweighofer et al. (2008), who also used ATD, ATL and BAL conditions, reported that ATD significantly increased the choice of the smaller sooner option by 4% and increased the discounting parameter compared to ATL. Interestingly, they found no effect of the ATL condition compared to the BAL condition. This pattern, however, was not consistently observed. An earlier study by the same group that used the same task with juice rewards (instead of money) during fMRI, found no effect of any intervention condition on discounting behaviour (Tanaka et al., 2007). They explain that time constraints inherent to fMRI experiments and the reduced number of trials in the 2007 study may explain the lack of behavioural results, compared to their 2008 study. Interestingly, a point they do not discuss is the fact that Tanaka et al. (2007) used primary rewards (squirts of an isotonic drink), while Schweighofer et al. (2008) used monetary rewards, which may have had an impact on discounting behaviour. A third study by the same group (Demoto et al., 2012) did not observe a significant change in discounting rates in the ATD vs. control condition. In summary, the data on whether 5-HT has a role on discounting rates, if delays are experienced during the task, are inconclusive. One explanation may be that all these studies suffer from small sample sizes, ranging from 12 to 20 participants. Another explanation for the inconclusive findings is related to the tryptophan intervention, which only has a moderate effect on the 5-HT system and acts rather unspecific on the whole brain compared to the measures used in animal studies described below. Therefore, the effect sizes elicited by tryptophan interventions may be too small to be measured in smaller samples.

It should be noted that we were not able to account for individual differences in the make-up of the 5-HT system, namely the role of 5-HT receptors, which have been shown both to play a role

in intertemporal choice and to interact with tryptophan interventions. For example, Faulkner et al. (2014) used positron emission tomography (PET) and provided preliminary evidence that increased temporal discounting was associated with reduced 5-HT1A receptor availability in the left hippocampus, probably related to prospective memory ability. A second study by the same group (Faulkner et al., 2017) showed that peripheral measured baseline levels of 5-HT1B receptor mRNA (a proxy for central 5-HT1B receptor mRNA levels) modulated the effect of ATD in a risky choice task. Specifically, 5-HT level changes in the depletion (compared to the sham) condition and higher 5-HT1B receptor mRNA levels were associated with increased gambling and increased sensitivity to larger wins. Very interesting for us is that they did not find an association with serotonin transporter mRNA levels, which suggests that the transporter is indeed not strongly involved in temporal and risky choice and highlights the importance of taking other markers of the 5-HT system (i.e. 5-HT1A, 5-HT1B receptors) into account.

Importantly, animal studies clearly showed a significant involvement of the 5-HT system on discounting behaviour in a way that substantially reduced brain 5-HT levels (by means of i.e. neurotoxin 5,7-DHT, which destroys 5-HT neurons when injected into the raphe nuclei, or drugs such as the 5-HT synthesis inhibitors pCPA via intraperitoneal injection) increased choices for the immediately available reward option (J. Bizot, Le Bihan, Puech, Hamon, & Thiebot, 1999; Denk et al., 2005; Mobini, Chiang, Al-Ruwaitea, et al., 2000; Wogar et al., 1993). In contrast, elevated 5-HT levels by means of serotonergic agents such as fluoxetine, fluvoxamine, citalopram, clomipramine and dexfenfluramine, increased choices for the delayed reward (J. Bizot et al., 1999; J. C. Bizot et al., 1988; Poulos et al., 1996). Moreover, recent research using optogenetic approaches showed that increased 5-HT firing promotes waiting behaviour (Fonseca et al., 2015; Xu, Das, Hueske, & Tonegawa, 2017). These data indeed support computational models suggesting that 5-HT modulates opportunity costs that are guiding temporal discounting (Boureau & Dayan, 2011; Cools et al., 2011). Hence, our null findings do not automatically falsify the predictions of these frameworks, but rather suggest that our and similar task designs with delays on the order of weeks or months capture a more abstract facet of discounting that may be not influenced that much by 5-HT. As such tasks demand human cognition, they cannot be well represented by animal studies.

Furthermore, we did not observe a direct effect of *5-HTTLPR* on ITeCh. To our knowledge, this is the first study that investigated the relationship between *5-HTTLPR* and ITeCh in humans. We could not replicate the results of Jedema et al. (2010) in macaques, who found stronger temporal discounting in L/L compared to L/S animals. However, given their sample size of four

macaques per group, the reported effect should be interpreted cautiously. One may argue that the number of trials used to estimate the discounting rate in our study was rather small and might result in low reliability of the discounting parameter, which might finally reduce statistical power of our study. Our data indicate, however, that the parameter estimation showed strong convergence after already 25 trials and are robust until the last trial (supplemental material, Fig. S5). Therefore, we believe that due to the adaptive Bayesian algorithm used in this task, we have reliably estimated discounting rates after 30 trials. Genotype did also not interact with the tryptophan interventions as reported in previous studies (Blair et al., 2008; Marsh et al., 2006). Therefore, it seems that *5-HTTLPR* does not have a significant association with discounting in our type of ITeCh task. However, note the limitation that we did not investigate the adenine-guanine single nucleotide polymorphism within *5-HTTLPR* (rs25531) that creates a triallelic locus (S, L_A, L_G) where the L_G variant shows similar activity as the S-allele (Hu et al., 2006). This may have obscured a potential effect of *5-HTTLPR* on temporal discounting or the BOLD response.

On the neural level, patterns of task-related activity observed by us are similar to those reported by Ballard and Knutson (2009), showing positive correlations of reward-related processing areas (medial frontal gyrus, ventral striatum) with future amount magnitude and negative correlations of delay duration with clusters in frontal, parietal and temporal brain regions. With respect to 5-HT, we did not observe an effect of the tryptophan intervention on the BOLD signal, neither for the parametric amount effect, nor for the parametric delay effect. One may argue that there are no findings to be expected given the lack of change in discounting rates, but for example the study by Tanaka et al. (2007) showed that the relationship between brain activity and behaviour can be complicated. They report that under ATD activity in the ventral striatum predicted rewards at shorter time scales (sooner offer) and, under ATL, activity in the dorsal striatum predicted rewards at longer time scales (later offer). This finding may be more closely related to the dynamic nature of the task, emphasizing the relevance of time scales (and how participants process delays). We, however, did not see any such effects on our time scales.

3.4.1. Conclusion

We investigated the role of 5-HT in the context of a computational framework, which suggests that 5-HT (together with DA) modulates opportunity costs of time and thereby alters temporal discounting. In contrast to its prediction that higher tonic 5-HT levels should reduce and lower tonic levels should increase the discounting rate, using two acute tryptophan interventions

(ATD/ATL) and a control condition (BAL), we did not observe any changes as measured with our ITeCh task. Furthermore, *5-HTTLPR* variants, which are supposed to regulate 5-HT transmission, were also not significantly associated with temporal discounting, and did not interact with tryptophan intervention. In line with the behavioural results, there were no effects of intervention or genotype on the BOLD activity during ITeCh. We speculate that our null finding might be related to the task design in a way that hypothetical choices, for example money offered with delays on a longer timescale, are not affected strongly by 5-HT in contrast to task where delays can be acutely experienced (i.e. shorter timescale). Future studies should clarify the involvement of 5-HT on the opportunity costs with respect to intertemporal choice using dynamic temporal discounting tasks and larger sample sizes to increase power.

4. Study 3 – Connection Failure: Differences in White Matter Microstructure are associated with *5-HTTLPR* but not with Risk-Seeking for Losses

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Abstract

In a previous study, we found for the *5-HTTLPR* genotype higher risk-seeking for losses in S/S vs. L/L carrier, which could not be explained by acutely changed central serotonin levels. This finding alternatively may be the result of reduced top-down control from the frontal cortex due to altered signal pathways involving the amygdala and ventral striatum. The serotonergic system is known to be involved in neurodevelopment and neuroplasticity. Therefore, the aim of this study was to investigate whether structural differences in white matter can explain the differences in risk-seeking behaviour that we observed in our previous study and whether *5-HTTLPR* groups differ in their white matter microstructure. These differences can be detected using diffusion-weighted imaging. We assumed lower structural connectivity in S/S compared to L/L carrier, and a negative relationship between risk-seeking for losses and connectivity. We used diffusion-weighted imaging to compute diffusion parameters for the frontostriatal and uncinate tract in 175 individuals (39 S/S, 80 S/L, 56 L/L). Results showed no significant relationship between diffusion parameters and risk-seeking for losses. Furthermore, we did not find significant differences in diffusion parameters of the S/S vs. L/L group. There were only group differences in the frontostriatal tract showing stronger structural connectivity in the S/L group, which is also reflected in the whole brain approach. Therefore, the data do not support our hypothesis that the association between *5-HTTLPR* and risk-seeking for losses is related to differences in white matter pathways implicated in decision-making.

4.1. Introduction

Understanding the neurobiological basis of decision-making under risk is an important target in the neuroeconomics community. Since Kahneman and Tversky (1979) showed with their prospect theory that individuals do not behave rationally when making choices involving risks, but instead show a pattern termed the *reflection effect*. It describes the observation that, when offered a smaller but certain amount of money and a larger but probabilistic amount to gain, individuals are risk-averse, i.e. they prefer the safe option. The opposite pattern, hence *reflection effect*, is shown when the offers are about losing money, either a certain smaller amount or a larger but probabilistic amount. Here individuals usually behave more risk-seeking, i.e. they choose the probabilistic offer more often than the safe option.

This reflection effect has been suspected to stem from emotional responses that bias choices to be more risk-averse or risk-seeking. Indeed, using a risky choice paradigm where offers were framed either as gains or losses. De Martino, Kumaran, Seymour, and Dolan (2006) found heightened amygdala activation when choices were made in agreement with the reflection effect (sure option in the gain domain, risky option in the loss domain) compared to the opposite behaviour. Further evidence from a mixed gambles task showed that the ventral striatum (VS) codes for the expected value of probabilistic choices in the gain domain and the amygdala for expected value in the loss domain (Yacubian et al., 2006). In general, the striatum and medial parts of the frontal cortex are known to carry valuation signals by integrating outcome related information such as magnitude, probability and delay (for reviews see Peters & Büchel, 2011; Rangel, Camerer, & Montague, 2008) and some evidence suggests that the valuation process may be influenced by the amygdala (Gottfried, O'Doherty, & Dolan, 2003). Further evidence for a role of the amygdala in loss related decision-making is provided by De Martino, Camerer, and Adolphs (2010) who showed that amygdala mediates loss aversion behaviour possibly by computing an arousal signal related to the prospective monetary loss. Moreover, the amygdala has strong connections to frontal brain regions and recent studies showed that its activity is regulated via inhibitory top-down control by the ventromedial prefrontal cortex (vmPFC) and a loss of this control can result in potentiated amygdala activity (Motzkin, Philippi, Wolf, Baskaya, & Koenigs, 2015). Therefore, the question arises whether the inhibitory top down control is reflected in the structural makeup of fibre bundles connecting the vmPFC with brain regions such as the amygdala and VS.

Previous research suggests that the *5-HTTLPR*, a natural occurring genetic variation in the promoter region of the gene (SLC6A4) coding for the serotonin transporter (5-HTT), influences neuronal signalling between the amygdala, striatum and vmPFC/OFC (Hariri & Holmes, 2006). The 5-HTT is responsible for the reuptake of serotonin (5-HT) from the synaptic cleft to the presynaptic nerve terminal and hence regulates extracellular 5-HT levels. 5-HTT availability and regulatory activity is also very important during the development of the central nervous system as 5-HT influences neuronal plasticity, proliferation and differentiation (for a review see Persico, Kalueff, & LaPorte, 2010). *5-HTTLPR* regulates the transcriptional efficiency (and hence transporter availability) in a way that one allelic variant with 14 repeats (short or S-allele) results in lower transcriptional efficiency and the other variant with 16 repeats (long or L-allele) results in higher transcriptional efficiency and finally, 5-HTT availability. PET imaging with the radioligand [^{11}C]DASB revealed a high density of 5-HTT in the striatum, moderate to high in the amygdala and moderate in the vmPFC (Kobiella et al., 2011; Kranz, Kasper, & Lanzenberger, 2010).

The functional relevance of this genotype was demonstrated in a study by Roiser et al. (2009) who used a risky choice fMRI task with a gain and loss frame for the presented offers in participants either homozygous for the L_a-allele or the S-allele of the *5-HTTLPR* and tested for differences in choice behaviour and brain activation between the genotype groups. Although they did not find significant differences in choice behaviour, they observed increased amygdala activation only in the S/S group when they chose according to the frame effect compared to when they chose contrary to it. The L_a/L_a group did not show such an effect. An additional functional connectivity analysis using a psycho-physiological interaction analysis (PPI) revealed an increased amygdala-PFC coupling only in the L_a/L_a group when making choices counter to the frame effect. Increased amygdala activation, reduced amygdala-PFC coupling and reduced amygdala grey matter volume in S-allele carrier compared to L/L individuals have also been shown in the context of negative emotional stimuli (Kobiella et al., 2011; Pezawas et al., 2005). These findings indicate an important functional role for the amygdala in decision-making and emotion processing. Therefore, the reduced functional coupling and concurrent increase in amygdala activation observed in S-allele carrier may be due to a compromised cortico-amygdala pathway (Hariri & Holmes, 2006).

In support of this interpretation, a study by Klucken et al. (2015) used DTI to investigate white matter microstructural properties of the uncinate tract, a fibre bundle that connects the temporal lobe (including the amygdala) with the inferior frontal lobe (i.e. vmPFC/OFC) together with a

fear conditioning paradigm to measure amygdala reactivity across *5-HTTLPR* groups in 107 participants. They found increased amygdala activation in S-allele carrier compared to the L/L group as well as increased fractional anisotropy, a measure of structural connectivity, in the S-allele participants, which they interpreted as elevated bottom-up control. There are, however, two other studies (Jonassen et al., 2012; Pacheco et al., 2009) that found the opposite result, i.e. reduced fractional anisotropy for S-allele carrier. It should be noted that the former study only tested 33 and the latter 37 females, which makes it difficult to draw a strong conclusion based on their findings

Another important tract that has been of high interest in the realm of decision-making research is the frontostriatal (also termed accumbofrontal) tract, which connects the VS with the vmPFC (Rigoard et al., 2011). Especially in the delay discounting domain, several studies reported a negative relationship between FA values of this tract and temporal discounting rates in young adults (Peper et al., 2013) and developing populations in the age range of 8-25 years (Achterberg, Peper, van Duijvenvoorde, Mandl, & Crone, 2016; Olson et al., 2009). These studies suggest a relationship between the structural properties of the frontostriatal tract and delay discounting. However, little is known about how 5-HTTLPR modulates the structural properties of this tract and the relationship of this modulation with probabilistic choice.

In an earlier study, we found a significant relationship between *5-HTTLPR* and risk-seeking for losses (but not for risk-aversion for gains). Specifically, individuals homozygous for the S-allele were more risk-seeking compared to heterozygous and individuals homozygous for the L-allele. This effect was not present when we acutely manipulated 5-HT levels (Neukam et al., 2018) and we speculated that *5-HTTLPR* related differences in white matter structure may account for our finding.

Therefore, the aim of this study is to investigate whether our gene-behaviour association can be explained with differences in individual white matter connectivity. To this end, based on the literature, we focused on the uncinate and frontostriatal fasciculus as a priori volumes of interest as they are most likely to be involved in modulating decision-making and be modulated by *5-HTTLPR*. We assumed increased risk-seeking for losses in S/S carrier to be driven by reduced top-down control from the vmPFC resulting in a stronger emotional response towards losses and, possibly, altered evaluation processes in the striatum. Therefore, we expect reduced structural connectivity of the uncinate and frontostriatal tract in S/S compared to L/L genotype individuals and a negative relationship between the structural connectivity (indicated by higher

FA, AD and lower MD, RD) and risk-seeking for losses scores. Finally, we use an exploratory voxel-based approach of the whole brain white matter to investigate possible relationships between other fibre bundles, genotypes and behaviour.

4.2. Methods

4.2.1. Participants

This study is part of two larger projects that investigated the role of dopamine and serotonin on meta-control parameters and brain function (Deza-Araujo et al., 2019; Kroemer et al., 2019; Neukam et al., 2018). The recruitment was done via standardized invitation letters sent to addresses based on a random sample stratified by sex and age (20-40 years), which were provided by the residential registry. All interested individuals were screened and excluded if one of the following criteria applied: pregnancy; not fulfilling the common criteria for MR safety; a current somatic disease requiring medical treatment; any psychiatric disorders that required pharmacological treatment within the last year; and a lifetime history of one of the following conditions (for ICD-10): organic psychiatric disorders (F0), opiate, cocaine, stimulants, hallucinogens, inhalants or poly-substance dependence, schizophrenia or related personality disorders (F2), and affective disorders (F3). Participants who passed the screening were invited to the study; their visual acuity was checked to ensure that it was at least 0.8. In total 611 participants completed a baseline visit, in which blood was taken to be genotyped for the *5-HTTLPR* and stored at -81°C until further processing. Risk-seeking for losses was measured with a probability discounting for losses task using the value-based decision-making (VBDM) battery (Pooseh, Bernhardt, Guevara, Huys, & Smolka, 2018). Afterwards, participants were re-invited to take part either in the dopamine or serotonin project during which diffusion weighted images were acquired. The local ethics review board of the Technische Universität Dresden approved of the study protocols and all participants gave written informed consent in line with the Declaration of Helsinki.

4.2.2. Probability discounting for losses (PDL) task

In this task, participants had to choose between two offers: a smaller certain loss or a larger probabilistic loss, both simultaneously presented. All offers presented were randomly shown on the left or right side on the computer screen, and the chosen offer was indicated with a red frame. Participants were informed beforehand that one of their choices in every task would be selected randomly and deducted from the total balance they could accumulate during the

baseline visit. During the task, they did not get feedback about the outcomes of each choice. Based on individual choices, the discounting rate (*k*) was estimated assuming hyperbolic discounting, following the formula:

$$V = \frac{L}{(1+k\Theta)}$$

where Θ = (1 – P)/P is the transformation of reward probability P (2/3, 1/2, 1/3, 1/4, and 1/5) to odds against winning. The loss, L, ranged from –5 Euro to −20 Euro. The task consisted of 30 trials. In all tasks, the likelihood of choosing between the two offers follows a softmax probability function in which β>0 serves as a consistency parameter such that its large values correspond to a high probability of taking the most valuable action. The algorithm starts from liberal prior distributions on the parameters and, after observing a choice at each trial, updates the belief about the parameters using the Bayes' rule P(k, β | choice) ∞ P(choice| k, β)P(k, β) to find offers close at the individual indifference point. The estimated *k* parameter from the final trial best explains choice behaviour with high *k* values indicating increased risk-seeking as higher but probabilistic losses are discounted and hence preferred over smaller, certain losses. A detailed description of the mathematical framework is reported in Pooseh et al. (2018). All tasks were implemented in MATLAB (Release 2010a, The MathWorks, Inc., Natick, MA, USA) and the Psychtoolbox 3.0.10 based on the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997).

4.2.3. Genotyping

The collected blood samples were sent to the Central Institute of Mental Health in Mannheim, Germany, to perform the genotyping for the *5-HTTLPR*. The exact procedure is described elsewhere (Dukal et al., 2015). Due to the failure to take blood from nine participants, blood samples from 602 participants were available for genotyping. The observed allele frequency was 39.9% for S and 60.1% for L, with the following genotype groups: 99 S/S, 283 S/L, and 220 L/L. The allele and genotype frequencies did not deviate significantly from the Hardy-Weinberg equilibrium (χ^2 = 0. 2461, df = 1, p = 0.62).

4.2.4. Imaging

Diffusion-weighted images (DWI) were acquired on a 3 Tesla Magnetom TrioTim scanner (Siemens Healthcare GmbH, Erlangen, Germany), equipped with a 32-channel head coil. In total 36 transverse scans, consisting of 4 non diffusion-weighted (B0) and 32 non-collinear

diffusion-weighted images with a b1000 s/mm² factor, were obtained. Parallel imaging was realized with a GRAPPA factor = 2 while the other parameters were as follows: repetition time (TR) = 9200ms; echo time (TE) = 92 ms; a basis resolution of 128x128x72 mm³ with 2.1 mm isotropic voxels (no gap); field-of-view (FOV) = 275x275 mm. Additionally, a high-resolution T1-weighted magnetization prepared rapid acquisition gradient echo (MP-RAGE) image for normalization, anatomical localization as well as screening for structural abnormalities by a neuroradiologist (TR: 1900 ms; TE: 2.26 ms; flip angle: 9°; FOV: 256 × 256 mm; 176 sagittal slices; voxel size: 1 × 1 × 1 mm) was acquired. Preprocessing of the DWI data included motion and eddy current correction using FSL (version 5.0.11) eddy (Andersson & Sotiropoulos, 2016), as well as the *–repol* setting to detect and replace slices that can be considered as outliers using default parameter (Andersson, Graham, Zsoldos, & Sotiropoulos, 2016) within the Nipype framework (Version 0.9.2, Gorgolewski et al., 2011). All 221 volumes were visually inspected for artefacts. In total, 38 data sets had to be excluded: 26 because of strong absolute rotation ≥ 1° along the x, y, or z axis, 2 showed abnormally large ventricles, and 10 suffered from severe distortion artefacts. The preprocessed images were then loaded into the ExploreDTI toolbox (Leemans, Jeurissen, Sijbers, & Jones, 2009) and the diffusion tensor model was fitted to the data using the RESTORE algorithm (Chang, Jones, & Pierpaoli, 2005). Afterwards, the DT matrices of interest were computed: FA, mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) and deterministic whole brain tractography (Basser, Pajevic, Pierpaoli, Duda, & Aldroubi, 2000) was performed (minimum FA = 0.2, minimal fibre length = 30 mm, maximal fibre length = 300 mm, maximum angle = 30°, cubic interpolation).

4.2.5. Frontostriatal and uncinate fiber tract selection and volume of interest (VOI) generation

4.2.5.1. Uncinate fasciculus

To obtain a VOI for the uncinate fasciculus, we employed a similar method that has been reported by Schaeffer et al. (2014). To this end, we used the TBSS pipeline to warp all individual DTI images to MNI space (FMRIB58 template). The warped FA images were thresholded to include only voxels with a value of at least 0.2 or above. We took the uncinate fasciculus from the John Hopkins University white matter tractography probability atlas (Hua et al., 2008) and thresholded the probabilities to greater or equal 5% to exclude less likely voxels. Finally, each normalized and thresholded FA image was combined with thresholded probabilistic tract map and the mean FA skeleton computed during the TBSS procedures

described above. The resulting VOIs were then retransformed to native space and applied to the DTI images to extract the parameters of interest. A VOI is shown in Fig. 6(a).

4.2.5.2. Frontostriatal tract

As the frontostriatal tract is not yet part of white matter atlases, we used a region of interest (ROI) approach, combined with the individually computed tractograms. First, we generated a ROI of the striatum by combining the accumbens, putamen and caudate parts from the Harvard-Oxford subcortical atlas implemented in FSL. Next, we used regions (Frontal_Sup_Orb, Frontal_Med_Orb, Rectus) from the automated anatomic labeling atlas (Tzourio-Mazoyer et al., 2002) to create a vmPFC/OFC mask. In order to transform the ROIs from MNI standard space to individual diffusion space, a series of computations were performed. The first step was to register the individual T1 image to MNI space using the *flirt* and *fnirt* algorithms thereby obtaining the non-linear transformation coefficients of interest. The next aim was to average and skull-strip the B0 images and register the averaged image to the individual T1 image using *flirt*. The obtained transformation matrix was then used with *applywarp*, together with the non-linear transformation coefficients to warp the B0 image to MNI space. As we were interested in having the inverse matrices to transform the ROIs from MNI to diffusion space, we used to *convert_xfm* and *invwarp* operations to invert the transformations from MNI to T1 space and from T1 to diffusion space. The inverted matrices and the *applywarp* command were then used to transform the striatum and vmPFC/OFC masks from MNI to diffusion space.

The individual tractograms and transformed ROIs were next loaded into TrackVis (version 0.6.1; Wang & Wedeen, 2015) and streamlines that pass from the striatum to the vmPFC/OFC or vice versa were generated. Exclusion masks were individually set on the mid sagittal plane, on the coronal plane at the splenium of the corpus callosum, and on the axial plane on the level of the anterior temporal gyrus. If necessary, single spurious streamlines were additionally manually removed. The resulting tracts were then exported as nifti files.

A prerequisite for the creation of a group template was the employment of the FSL tract-based spatial statistics (TBSS) pipeline (Smith et al., 2006), which warps individual FA maps to MNI space (the FMRIB58 template provided by FSL), averages all FA images to compute a mean FA image, which is then reduced to a skeleton, based on voxels from the nearest tract centre. In a next step, all tracts were non-linearly warped to the MNI template, registered to the FA skeleton using the *tbss_non_fa* command from TBSS and binarized. Finally, all tracts were summed up into one nifti, normalized by the number of participants, and thresholded to contain

only voxels that exist in at least 50% of the sample, and binarized again. This group VOI was then retransformed to native space and applied to the DTI metrics of interest (FA, MD, AD, RD) to extract the tract related metrics. A VOI is depicted in Fig. 6(b).

4.2.6. ROI statistical analysis

To address the question whether there is a relationship between *5-HTTLPR* groups, white matter structure and risk-seeking for losses, we used a multivariate analysis of covariance (MANCOVA) for each tract with FA, AD, MD and RD as dependent variables with genotype (S/S, S/L, L/L) as group factor and logarithm of *k* from the PDL task as covariate of interest, as well as sex and age as control variables. We set our statistical threshold of significance at $p < .05$.

4.2.7. Whole brain analysis

To explore potential effects of *5-HTTLPR* and risk-seeking for losses in other regions of the brain, we used the TBSS pipeline described above to skeletonize all FA, MD, AD, and RD images. We used voxelwise non-parametric statistical analyses based on 10.000 random permutations and the threshold-free cluster enhancement (TFCE) approach to test for the main effect of genotype (S/S, S/L, L/L), the main effect of risk-seeking for losses and interaction effects while controlling for sex and age. Additionally, age and sex were demeaned and entered as covariate regressors as they were found in previous studies to be related to the DTI parameters. We assumed significance at a family-wise error corrected p-value of $< .05$. Classification of tracts the clusters belong to was performed with the JHU White Matter Tractography Atlas (Hua et al., 2008). To further explore the contribution of each genotype to all significant clusters, binarized masks were generated from them and the four DTI parameters were extracted and averaged across cluster voxels, separately for each parameter. In a next step, a multivariate ANOVA was conducted with the four DTI parameters as dependent variables and genotype as predictor.

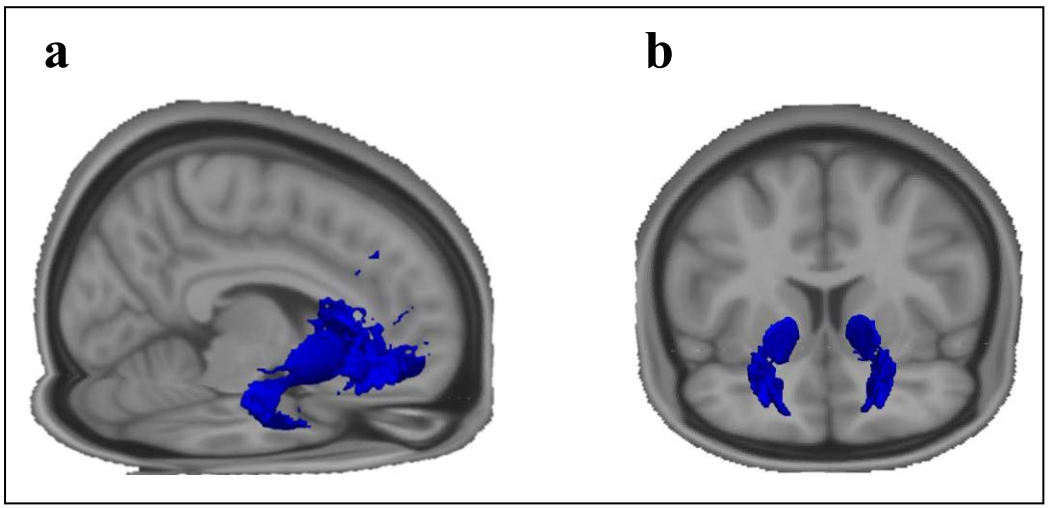

Fig. 6 Volumes of interest of the (a) uncinate tract and (b) frontostriatal tract. Both volumes were created in standard space where the uncinate tract was created based on an atlas template and the frontostriatal tract was created based on individual tractography. Only voxels in deep white matter were analyzed based on the FA skeleton created with TBSS. See section 4.2.5. for details.

4.3. Results

4.3.1. Sample information

Eight participants out of the 183 had missing PDL data resulting in 175 data sets for analysis. This sample consisted of 39 (14 females) S/S carrier, 56 (12 females) S/L carrier and 80 (35 females) L/L carrier demonstrating an overall greater number of males than females ($\chi^2 = 7.252$, df = 2, p = .027). Age was similarly distributed among genotype groups ($F_{2,169} = 3.027$, p = .051): S/S (males: 32.8 ± 6.1, females 31.4 ± 5.5 years), S/L (males: 34.8 ± 4.3, females 34.9 ± 4.7 years), S/L (males: 32.8 ± 6.1, females 31.4 ± 5.5 years), and L/L (males: 33.8 ± 5.1, females 32.3 ± 5.9 years).

4.3.2. Tract analysis

To test our first hypothesis, we used a multivariate analyses of variance (MANOVA) separately for each tract and investigated simple linear contrasts to compare the homozygous S- and L-allele groups, controlling for sex and age. For the second hypothesis, we used partial Pearson correlations to investigate the relationship between the *k* values (on log scale to approximate a normal distribution) from PDL and the DTI parameters, controlling for *5-HTTLPR*, sex and age.

4.3.2.1. Association between 5-HTTLPR and the frontostriatal tract

The simple linear contrast analyses between S/S and L/L groups did not reveal any significant differences for each of the DTI metrics (all $p > .06$). See Table 7 and Fig. 7 for details. The results did also not change when we combined the S/S and S/L group.

However, the multivariate analysis showed significant main effects of genotype (Wilk's $\Lambda = 0.924$, $F_{6,330.000} = 2.222$, $p = .041$, $\eta_p^2 = .039$), sex (Wilk's $\Lambda = 0.953$, $F_{3,165.000} = 2.689$, $p = .048$, $\eta_p^2 = .047$) and age (Wilk's $\Lambda = 0.952$, $F_{6,330.000} = 2.222$, $p = .041$, $\eta_p^2 = .039$). For the main effect of genotype, an exploratory oneway ANOVA with Games-Howell post-hoc tests showed that S/L individuals had significantly higher FA values than L/L individuals ($M_{S/L} = 0.509 \pm 0.018$, $M_{L/L} = 0.498 \pm 0.024$, $p = .013$), S/S had higher AD values compared to S/L individuals ($M_{S/S} = 1.23\times10^{-3} \pm 2.22\times10^{-5}$, $M_{S/L} = 1.21\times10^{-3} \pm 3.01\times10^{-5}$, $p = .007$), while S/S and L/L carrier had higher MD ($M_{S/S} = 7.63\times10^{-4} \pm 2.00\times10^{-5}$, $M_{S/L} = 7.49\times10^{-4} \pm 2.22\times10^{-5}$, $p = .004$; $M_{L/L} = 7.62\times10^{-4} \pm 2.13\times10^{-5}$, $M_{S/L} = 7.49\times10^{-4} \pm 2.22\times10^{-5}$, $p = .002$) and RD values ($M_{S/S} = 5.28\times10^{-4} \pm 2.40\times10^{-5}$, $M_{S/L} = 5.15\times10^{-4} \pm 2.35\times10^{-5}$, $p = .024$; $M_{L/L} = 5.31\times10^{-4} \pm 2.85\times10^{-5}$, $M_{S/L} = 5.15\times10^{-4} \pm 2.35\times10^{-5}$, $p = .001$) compared to S/L carrier.

Furthermore, post-hoc independent sample t-tests demonstrated that the effect of sex was related to higher FA ($M_{males} = 0.506 \pm 0.019$, $M_{females} = 0.497 \pm 0.024$, $t_{173} = -2.529$, $p = .012$) lower MD ($M_{males} = 7.55\times10^{-4} \pm 2.14\times10^{-5}$, $M_{females} = 7.65\times10^{-4} \pm 2.22\times10^{-5}$, $t_{173} = 2.962$, $p = .003$) and RD ($M_{males} = 5.31\times10^{-4} \pm 2.44\times10^{-5}$, $M_{females} = 5.33\times10^{-4} \pm 2.95\times10^{-5}$, $t_{173} = 2.888$, $p = .004$), but not AD ($M_{males} = 1.22\times10^{-3} \pm 2.76\times10^{-5}$, $M_{females} = 1.23\times10^{-3} \pm 2.07\times10^{-5}$, $t_{173} = 1.593$, $p = .113$) in males compared to females. Finally, exploratory Pearson's correlation showed that age was significantly negatively correlated with AD ($r = -.219$, $p = .004$), but not with FA ($r = .005$, $p = .948$) nor MD ($r = -.143$, $p = .059$) nor RD ($r = -.073$, $p = .336$).

Table 7 Results of the simple linear contrast analyses

	S/S		L/L		Contrast estimate	p-value
	Mean	SD	Mean	SD		
Frontostriatal						
FA	0.50	0.02	0.50	0.02	-3.80E-03	.369
AD	1.23E-03	2.22E-05	1.23E-03	2.22E-05	-9.40E-06	.060
MD	7.63E-04	2.00E-05	7.62E-04	2.13E-05	-2.16E-06	.611
RD	5.28E-04	2.40E-05	5.31E-04	2.85E-05	1.46E-06	.780
Uncinate						
FA	0.47	0.01	0.46	0.02	-0.004	.278
AD	1.21E-03	2.44E-05	1.21E-03	2.15E-05	-1.76E-06	.715
MD	7.76E-04	1.95E-05	7.79E-04	2.08E-05	1.944E-06	.639
RD	5.59E-04	2.08E-05	5.63E-04	2.50E-05	3.797E-06	.407

FA=Fractional Anisotropy, AD=Axial Diffusivity, MD=Mean Diffusivity, RD=Radial Diffusivity, SD=Standard Deviation

4.3.2.2. Association between 5-HTTLPR and the uncinate tract

Here, there was also no significant difference between the two homozygous groups with respect to the DTI parameters (all $p > .28$). Results are also shown in Table 7 and Fig. 7. Similarly, as above, combining the S-allele groups did not change the results significantly.

For this tract, the multivariate analysis showed no significant main effects of genotype (Wilk's $\Lambda = 0.958$, $F_{6,330.000} = 1.192$, $p = .310$, $\eta_p^2 = .021$), but a significant effect of sex (Wilk's $\Lambda = 0.952$, $F_{3,165.000} = 2.786$, $p = .042$, $\eta_p^2 = .048$) and age (Wilk's $\Lambda = 0.938$, $F_{6,330.000} = 3.643$, $p = .014$, $\eta_p^2 = .062$). Exploratory post-hoc independent sample t-tests demonstrated that the effect of sex was related to a slightly higher FA ($M_{males} = 0.469 \pm 0.015$, $M_{females} = 0.463 \pm 0.020$, $t_{96.101} = -2.024$, $p = .046$) lower AD ($M_{males} = 1.20\times10^{-3} \pm 2.59\times10^{-5}$, $M_{females} = 1.21\times10^{-3} \pm 2.17\times10^{-5}$, $t_{173} = 2.828$, $p = .005$), MD ($M_{males} = 7.71\times10^{-4} \pm 1.98\times10^{-5}$, $M_{females} = 7.82\times10^{-4} \pm 2.23\times10^{-5}$, $t_{173} = 3.550$, $p = <.001$) and RD ($M_{males} = 5.54\times10^{-4} \pm 2.06\times10^{-5}$, $M_{females} = 5.66\times10^{-4} \pm 2.64\times10^{-5}$, $t_{173} = 3.315$, $p = .001$) in males compared to females. For the age effect, Pearson's correlation showed that age was again significantly negatively correlated with AD ($r = -.242$, $p = .001$), but not with FA ($r = -.064$, $p = .397$) nor MD ($r = -.136$, $p = .073$) nor RD ($r = -.057$, $p = .455$).

4.3.2.3. Correlations between frontostriatal/uncinate tract and PDL

The results are visualized in Fig. 8. In brief, there were no significant correlations (all $p > .14$) between DTI parameters of each tract with PDL as shown in Table 8.

Table 8 Correlations of logk PDL with DTI parameters

DTI parameter	Pearson's r	p-value
Frontostriatal tract		
FA	-0.042	0.584
AD	-0.024	0.757
MD	0.004	0.956
RD	0.016	0.830
Uncinate tract		
FA	-0.104	0.170
AD	-0.111	0.142
MD	-0.020	0.798
RD	0.032	0.672

FA=Fractional Anisotropy, AD=Axial Diffusivity,
MD = Mean Diffusivity, RD=Radial Diffusivity

4.3.3. Whole brain analysis

The results for the main effect of genotype are summarized in Table 9. There were no significant clusters for the main effect of PDL, nor for the interaction with genotype.

Simple linear contrast analyses in the context of the MANOVA showed that S/S individuals had higher FA values ($M_{S/S} = 0.48 \pm 0.018$, $M_{L/L} = 0.47 \pm 0.017$, $p = .020$, Cohen's $d = -0.434$) and lower RD values ($M_{S/S} = 5.44\times10^{-4} \pm 2.21\times10^{-5}$, $M_{L/L} = 5.47\times10^{-4} \pm 2.46\times10^{-5}$, $p = .021$, Cohen's $d = 0.414$) compared to L/L individuals. Visual depictions of the main effect of genotype are shown in the supplemental material, Figs. S6/S7.

Table 9 Summary cluster map of the TBSS results for the main effect of genotype

Tracts	Side	Peak voxel (MNI)			F-Statistic	Cluster size > 100 (voxels)	Cluster p-value
		x	y	z			
FA							
SFOF	Left	-22	-2	19	19.5	10610	0.001
ILF	Right	45	-11	-27	14.2	6452	0.003
UNC, IFOF	Right	18	24	-12	13.8	669	0.015
AD							
Unclassified	Left	-10	-1	-14	17.3	12958	0.001
ILF	Right	40	-22	-21	11.5	1739	0.014
Forceps minor	Right	12	31	8	10	1522	0.028
UNC, IFOF	Right	28	14	-10	9.84	786	0.03
Unclassified	Right	1	10	14	7.64	188	0.047
Forceps minor	Left	-12	29	-12	9.1	157	0.042
SLF	Left	-34	-37	21	10.3	141	0.038
ATR, IFOF	Right	23	26	23	5.94	141	0.047
MD							
ATR	Left	-11	-17	-2	18.1	31113	0.001
RD							
ATR	Left	-23	-2	17	18.8	14709	0.001
ILF	Right	45	-10	-28	15.3	6641	0.004
UNC, IFOF	Right	18	24	-12	13.4	209	0.039

FA = Fractional Anisotropy; AD = Axial Diffusivity; MD = Mean Diffusivity; RD = Radial Diffusivity; SFOF = Superior Fronto-Occipital Fasciculus; ILF = Inferior Longitudinal Fasciculus; UNC = Uncinate Fasciculus; SLF = Superior Longitudinal Fasciculus; ATR = Anterior Thalamic Radiation

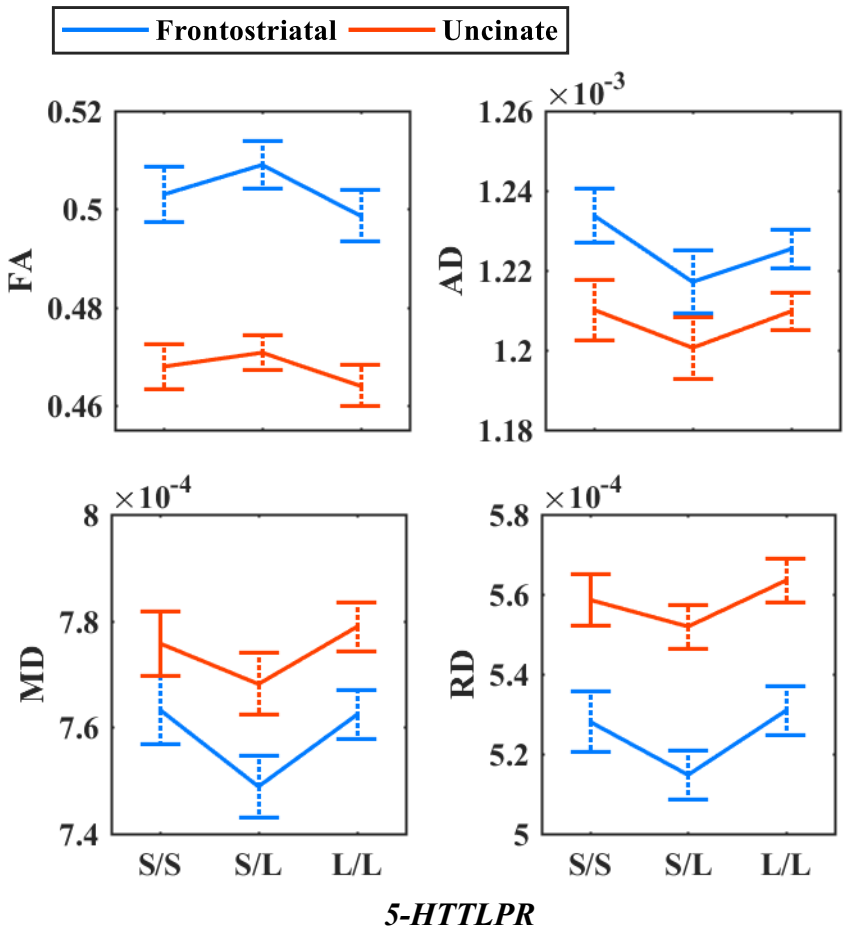

Fig. 7 Main effect of *5-HTTLPR* on DTI parameters: fractional anisotropy (FA), axial diffusivity (AD), mean diffusivity (MD) and radial diffusivity (RD). Error bars are bootstrapped with 10.000 iterations and denote 95% bias corrected and accelerated confidence intervals.

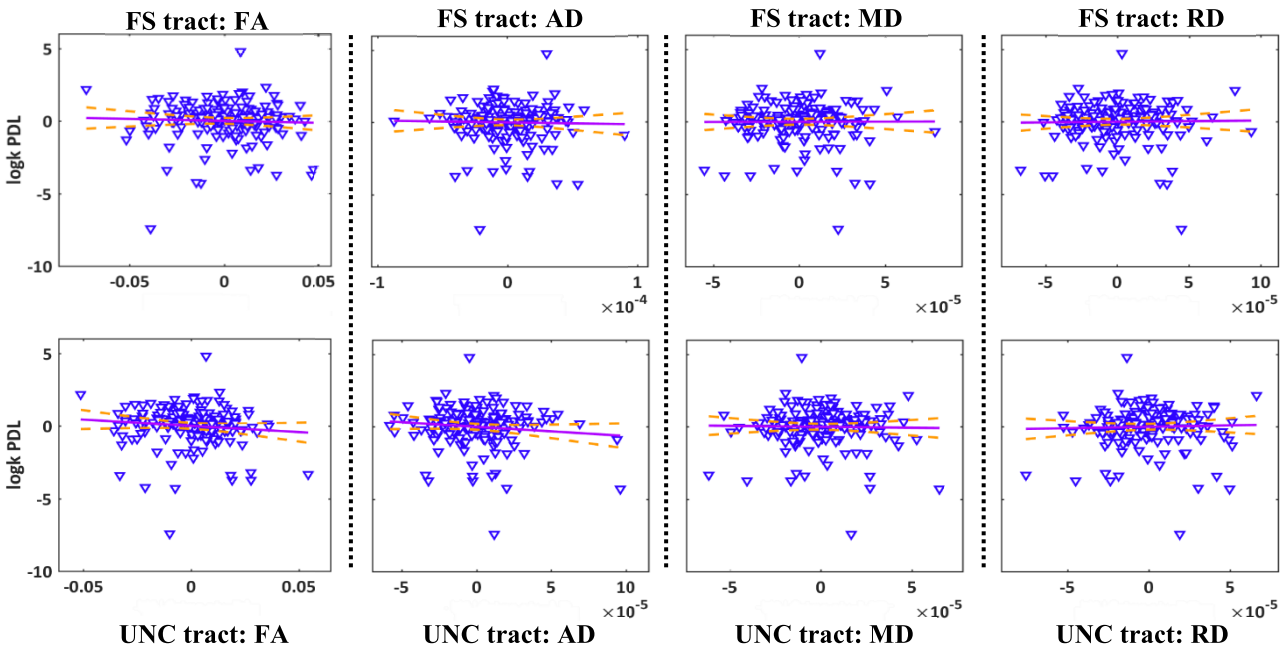

Fig. 8 Correlations between risk-seeking for losses (logk PDL) and DTI parameters of the uncinate (UNC) and frontostriatal (FS) tract: fractional anisotropy (FA), axial diffusivity (AD), mean diffusivity (MD) and radial diffusivity (RD). All DTI parameters are unstandardized residuals after controlling for *5-HTTLPR*, sex and age.

4.4. Discussion

The purpose of this study was to elucidate whether the association between risk-seeking for losses, as measured with a PDL task, and *5-HTTLPR* we observed earlier (Neukam et al., 2018) may be explained by differences in white matter connecting brain regions that are involved in value-based decision-making (vmPFC, VS, amygdala). Based on existing literature, we chose the frontostriatal and uncinate tract. The former connects the VS with the vmPFC and the latter the vmPFC with the amygdala. Interestingly, the frontostriatal tract has been implicated in decision-making behaviour, but not in *5-HTTLPR*, while the opposite is true for the uncinate tract. This study extends on these findings by examining the association of *5-HTTLPR* with both tracts and furthermore by investigating the relationship between the tracts and risk-seeking for losses, which has not been published before according to the authors knowledge.

The results of all data analyses can be summarized in two parts. First, the DTI parameters (FA, AD, MD, RD) are not related to the discounting rates of the PDL task, neither in the tracts of interest nor with whole brain white matter. Therefore, differences in white matter structure cannot explain risk-seeking for losses in our sample. Second, we did not find the expected linear relationship between genotype and DTI parameters (i.e. higher FA, AD and lower MD, RD for L/L compared to S/S carrier) neither in the frontostriatal, nor in the uncinate tract, nor in other

white matter bundles. Hence, we could not replicate previous findings indicating reduced structural connectivity in S/S compared to L/L carrier (Jonassen et al., 2012; Pacheco et al., 2009).

4.4.1. White matter and risk-seeking for losses

There are not many reports that studied the contribution of white matter microstructure to value-based decision-making. The majority of existing studies focused on intertemporal choice and found negative correlations between white matter microstructure (such as the frontostriatal tract) and delay discounting (i.e. higher structural connectivity and reduced temporal discounting rates) in longitudinal studies examining participants ranging between 8-26 years (Achterberg et al., 2016; Olson et al., 2009) and young adult populations ranging between 18-25 years (Peper et al. (2013); van den Bos, Rodriguez, Schweitzer, and McClure (2014); but see Hampton, Alm, Venkatraman, Nugiel, and Olson (2017) for an opposite finding). Much less is specifically known about the relationship between risk-seeking for losses and the uncinate fasciculus. The main motivation to select this tract was that it denotes an important pathway connecting the amygdala to the vmPFC. Research in humans and mice indicated that the frontal cortex regulates the amygdala by reducing its activation in the wake of negative events (Adhikari et al., 2015; Motzkin et al., 2015). Therefore, reduced structural connectivity may be associated with reduced top-down control, higher amygdala activity and, finally, increased risk-seeking for losses (De Martino et al., 2006).

However, our preliminary findings do not support the conclusion of the studies investigating the frontostriatal tract that higher impulsivity (steeper discounting) is associated with reduced structural connectivity in the context of risk-seeking for losses. It is tempting to speculate that age may be a reason for our null finding as all previous studies had much younger samples. Karlsgodt et al. (2015) for example showed that the frontostriatal tract microstructure (i.e. FA) increases steadily during childhood until the early twenties to stabilize and slowly decreases around the age of forty. Hence, we have not been able to capture developmental aspects of the decision-making related white matter, in contrast to the studies above, which may explain our null finding. Still, as there are currently no directly relatable data published it seems premature to draw a final conclusion on whether our finding is a true or false negative.

4.4.2. *5-HTTLPR* and white matter

Previous studies have shown interest in understanding the white matter microstructure of the uncinate fasciculus in relation to the *5-HTTLPR* because numerous studies using functional and morphometric measures suggest that S-allele carrier have increased amygdala activity (Hariri et al., 2002; Heinz et al., 2007), reduced grey matter volume (Kobiella et al., 2011; Pezawas et al., 2005) and reduced coupling of the amygdala to the frontal cortex (Pezawas et al., 2005) compared to L/L carrier. This is in line with the hypothesis that there is a gene-dose-effect, where the gene function increases with the number of L-alleles (Hu et al., 2006; Wendland et al., 2008). Such a relationship was found in two studies investigating the uncinate fasciculus that showed increasing FA values with the number of L-alleles (Jonassen et al., 2012; Pacheco et al., 2009). Due to the observation that S/S and S/L individuals have similar 5-HTT expression rates and also score similarly on behavioural measures such as trauma exposure (Goldman, Glei, Lin, & Weinstein, 2010), neuroticism (Lesch et al., 1996) and depressive symptoms (Neumeister et al., 2006), studies combine S-allele groups (S/S, S/L) and compare them to L/L carrier. Such an approach was conducted by Klucken et al. (2015) who found the opposite pattern of FA values (S > L/L), but did not find any association for genotype and FA in a replication study (Klucken et al., 2018). This latter finding is in line with our observation that genotype does not significantly affect FA nor AD, MD and RD in the uncinate tract and the fact that Jonassen et al. (2012) and Pacheco et al. (2009) only analysed 33 and 37 females, respectively limits the generalizability of their studies. Additionally, Klucken et al. (2015) found the opposite in 100 participants containing both sexes in a first study and no genotype effect in their replication study including 114 participants and finally our null finding with 175 participants supports the notion that the genotype effect is either very small or depends on other presently unknown third variables, following the arguments brought forward by Klucken et al. (2018).

We also did not find the expected genotype effect, reduced structural connectivity in S/S compared to L/L carrier, in the frontostriatal tract. Instead we found non-linear effects of genotype in AD, MD and RD demonstrating less MD in S/L compared to S/S and L/L carrier, less AD in S/L compared to S/S carrier and lower RD in S/L compared to L/L carrier. These findings are not intuitive and are at odds with a gene-dose-effect. An explanation may be a larger proportion of individuals showing molecular heterosis in our sample, a phenomenon that describes heterozygosity in a given genetic polymorphism can result either in a greater

expression (positive heterosis) or lesser expression (negative heterosis) of a phenotype compared to homozygosity and such observation may occur in up to 50% of all human genetic association studies (Comings & MacMurray, 2000). In case of *5-HTTLPR* there is research reporting such findings in the context of 5-HTT binding potential or 5-HTT availability where S/L individuals had lower scores compared to S/S and L/L (Little et al., 1998; van Dyck et al., 2004). Furthermore, Malmberg, Wargelius, Lichtenstein, Oreland, and Larsson (2008) reported that male S/L adolescents had higher scores for disruptive behavioural disorder and Steffens, Taylor, McQuoid, and Krishnan (2008) observed higher white matter volume lesions in geriatric depressed patients in comparison to the homozygous groups. Our results are in line with these observations but the mechanisms behind heterosis are not yet understood. Comings and MacMurray (2000) suggest three possible reasons: the first being an (inverted) U-shape function indicating that both too little or too much expression has adverse consequences and only intermediate expression is advantageous; the second being an independent third factor causing a hidden stratification of the sample such that in one set S/S carrier have the highest/lowest phenotypic expression and in the second set L/L carrier have the highest/lowest phenotypic expression. The third reason may be greater fitness in heterozygous individuals because they show a broader range of gene expression compared to the homozygous groups. Nevertheless, given that this is the first study reporting such a finding with DTI parameters in the frontostriatal tract, more studies are needed to support this finding.

4.4.3. Limitations

One possible limitation is that our diffusion-weighted imaging sequence was not sensitive enough to find correlations between the DTI parameters and risk-seeking for losses as well as the expected linear relationship with *5-HTTLPR*. However, despite the fact that we only collected data from 32 direction whereas newer sequences acquire data from twice our number or even more directions, we believe that our number of directions is sufficient to estimate the tensor model and, importantly, to replicate an often published finding that males show consistently higher FA and lower MD and RD compared to women in the frontostriatal and uncinate tract (while the results of AD are inconclusive), which is in line with previous findings that males have a higher structural connectivity compared to females in several brain regions (Menzler et al., 2011; van Hemmen et al., 2017; Westerhausen et al., 2004). Another limitation is that we could not use the triallelic *5-HTTLPR* model, which may have given us more information about the reliability of the heterosis effect.

4.4.4. Conclusion

Overall, we did not find a significant correlation between white matter parameter and risk-seeking for losses in two highly relevant fibre bundles, nor the expected association with respect to *5-HTTLPR*, which may have explained the genotype-behaviour finding in our earlier study (Neukam et al., 2018) and supports the idea of reduced top-down control in S/S compared to L/L individuals. Nevertheless, we found some evidence for the potential existence of heterosis in the frontostriatal tract that need validation from future studies. Additionally, more studies are needed to support our finding that white matter microstructure connecting the vmPFC with the striatum and amygdala is not related to differences in risk-seeking for losses.

5. General Discussion

The main purpose of this thesis was to contribute to the (large) discussion on how serotonin influences value-based decision-making (VBDM). To this end, the framework proposed by Cools et al. (2011) that describes an intuitive mechanism (the overall average outcome rate) through which central tonic 5-HT (and DA) act to influence a range of different decision-making tasks was used. Furthermore, a candidate gene approach was employed, which investigated *5-HTTLPR*, a naturally-occurring, single nucleotide polymorphism that has functional phenotypes both in structural development and signalling of the 5-HT system. Following this, the proposed framework was comprehensively tested using experimental and genetic approaches, as well as and four different tasks from our newly developed VBDM battery in one larger community sample. Finally, functional magnetic resonance imaging (fMRI) and diffusion-weighted imaging (DWI) were used to investigate serotonergic effects on the brain level.

5.1. Study 1 – Experimental and genetic effects of 5-HT on probabilistic choice

In *Study 1*, we sought to elucidate the influence of 5-HT on probabilistic choice. Based on the framework, reduced tonic 5-HT should increase the overall average expected outcome, shifting the reference point to make smaller gains appear to be losses and hence reduce risk-aversion for gains while increasing risk-seeking for losses and loss-aversion. On the genetic level, we assumed that S/S carriers behave as if they have higher tonic 5-HT levels compared to S/L and L/L carriers. In order to test our hypotheses, we conducted a genetic study with a larger sample of 611 participants and a pharmacological study in which a subsample of 112 individuals participated in a double-blind, randomized, cross-over design consisting of three conditions in which tonic 5-HT levels were manipulated with a dietary acute tryptophan intervention.

Our results showed that acutely changed 5-HT levels did not alter decision-making behaviour in our VBDM tasks. On the genetic level, there was a significant association of *5-HTTLPR* with risk-seeking for losses, indicating that S/S carriers demonstrating higher risk-seeking compared to S/L and L/L carrier. No associations in other tasks were found. Taken together, tonic 5-HT did not affect probabilistic choice and no significant *5-HTTLPR* group differences were evidenced for risk-aversion for gains and loss aversion in the mixed gambles task. Only for risk-seeking for losses was an expected effect noticed that was independent of the tryptophan

intervention. This suggests an independent mechanism of 5-HT that may be related to its effects on the structure and wiring of the brain during development.

5.2. Study 2 – Experimental and genetic effects of 5-HT on intertemporal choice and the brain

In *Study 2*, we investigated the pharmacological and genetic effects of 5-HT on intertemporal choice (ITeCh) both on the behavioural and brain level. Here, we hypothesized that reduced 5-HT levels increase the expected overall outcome rate and consequently make waiting more punishing. This should be reflected in an increased discounting rate. Furthermore, we expected a reduced activation of the valuation network to delayed amounts and a stronger deactivation of the cognitive control network to delay durations during fMRI. For the genetic part, we expected reduced discounting rates in S-allele compared to L/L-allele carriers. The study design and sample were the same as in *Study 1*.

On the behavioural level, we found no effects of 5-HT on temporal discounting rates and also no association of *5-HTTLPR* with temporal discounting. This was also reflected by the BOLD response of the brain that showed the expected network activations for amount and delay, but did not vary as a result of the tryptophan intervention. Our finding is at odds with evidence from animal studies that used shorter time scales (seconds) compared to the more abstract time scales we utilized (weeks and months) that were not immediately experienced. This may present an important difference as it raises the possibility that the perception and processing of longer durations are not directly influenced by 5-HT.

5.3. Study 3 – Genetic effects of 5-HT on white matter microstructure and risk-seeking for losses

In *Study 3*, we followed up on the results of *Study 1* in which a significant association between *5-HTTLPR* and risk-seeking for losses that could not be explained by acutely manipulated 5-HT levels was determined. There is a significant amount of evidence that suggests an involvement of white matter in decision-making and an association with *5-HTTLPR*. Hence, differences in the structure of white matter that connects brain regions essential for valuation and affective processing may explain the increased risk-seeking for losses behaviour present in S/S individuals. We hypothesized that S/S individuals are more vulnerable to the prospect of losing, which is accompanied by higher arousal due to reduced top-down control. This is

reflected in reduced structural coherence compared to L/L carriers in pathways connecting valuation and emotional processing regions (i.e. frontostriatal tract and uncinate fasciculus).

We did not observe such a relationship in our data as S/S carriers did not demonstrate significantly different white matter makeup compared to L/L carriers in the tracts of interest. Furthermore, the white matter microstructure was found to be unrelated to risk-seeking for losses in our sample. These findings suggest that the microstructure of these white matter fibre bundles are unlikely to explain risk-seeking for losses behaviour among S/S carriers.

5.4. Integrative results summary of all studies

The main conclusion from all studies is that 5-HT exerts only a limited influence on VBDM. In particular, the functional aspect that was pharmacologically manipulated changed neither probabilistic nor intertemporal choice to a significant degree. Although these findings do not automatically falsify the computational framework postulated by Cools et al. (2011) they certainly raise questions relating to its generalisability. The gene-behaviour association regarding risk-seeking for losses we observed in *Study 1* is plausible on a theoretical level, however it warrants more research regarding a biological mechanism as the results of *Study 3* do not explain this finding from the perspective of white matter structural connectivity. The pharmacological findings are in line with a review that summarized the effects of tryptophan interventions on reward and punishment related tasks (Faulkner & Deakin, 2014) noting little experimental support for 5-HT and probabilistic/intertemporal choice. This is surprising given the results of nonhuman animal models that present strong evidence for 5-HT in VBDM. Therefore, over the following sections important methodological differences between human and nonhuman animal studies that may account for the lack of findings in this thesis, as well as the limits of the candidate gene approach and potential advancements will be elaborated upon.

5.5. Global systemic serotonergic interventions may not be feasible in light of the complexity of the 5-HT system

In both *Studies 1* and *2*, tonic 5-HT levels were manipulated via an acute tryptophan intervention, yet did not result in significant behavioural changes as measured by the VBDM battery and ITeCh task. One straightforward explanation for this, would be that the intervention either did not work or was conducted incorrectly. From the blood samples we collected throughout each session it is plain that in the peripheral circulation system, the amount of tryptophan availability increased and decreased as expected. We have to accept this peripheral

marker as a proxy for central tryptophan processing as there is currently no feasible technique for measuring central tryptophan and 5-HT levels in humans. There is, however, some evidence from rodents receiving an oral acute tryptophan intervention that showed tryptophan and 5-HT levels in the brain increased or decreased depending on the amount of tryptophan used (Biskup et al., 2012; Stancampiano et al., 1997) even though the precise mechanisms have yet to be fully understood (van der Plasse, 2013; van Donkelaar et al., 2011).

Still, one important weakness of the tryptophan intervention as a means of manipulating tonic 5-HT levels in humans is that it works on a systemic level by either reducing or increasing 5-HT synthesis, most likely in a similar fashion throughout the brain. The implication is that there will be a desired net effect in 5-HT signalling despite compensatory mechanisms such as the well-known negative feedback loop involving the 5-HT1A autoreceptor and the diverse inhibitory and excitatory 5-HT receptor types (cf. section 1.6). A related assumption is that higher/lower tonic 5-HT levels produce the same net effect in all brain regions implicated in the decision-making circuit, which is a simplification that holds no ground in light of the complexity of the 5-HT system in terms of receptor diversity and circuitry. In this regard, the relationship between 5-HT and reward/punishment processing may be more complex than suggested by Cools et al. (2011). Over the following paragraphs, some of the research highlighting the complexity in explaining why a systemic intervention may not yield expected results, despite targeting the 5-HT system will be discussed.

5.5.1. The assumptions about the role of 5-HT in Cools et al.'s (2011) framework

An important assumption of the framework is that reward and punishment processing are represented by DA and 5-HT signalling and that they interact to form an expectation about the overall average outcome rate when available options are evaluated. For choices that include the constraint that one has to wait for a larger reward this is thought to be controlled by the opportunity costs of time, which is higher for high DA/low 5-HT levels promoting more impatient and vigorous behaviour, while lower for low DA/high 5-HT levels promoting waiting (and less vigorous behaviour). Similarly, for choices that include probabilistic outcomes, the framework suggests that DA and 5-HT control the reference point against which the smaller sure and larger probabilistic outcome are compared. Here, high DA/low 5-HT levels suggest an increased propensity for risk-seeking for gains and losses, as well as increased loss aversion, while the opposite pattern is observed for low DA/high 5-HT levels. The implication is that these neurotransmitter systems signal in opponency throughout the relevant brain areas whereas

DA signals the expected reward of a given option and 5-HT the expected punishment, with the net effect observed by the behavioural output.

There is partial evidence from some newer studies that used in-vivo techniques, such as single-unit recordings and microdialysis at the dorsal raphe nuclei, which show that 5-HT signalling is important for waiting for a reward during a sequential food-water navigation task (K. Miyazaki et al., 2011; K. W. Miyazaki, K. Miyazaki, & K. Doya, 2011). For example, an increase of tonic 5-HT firing was associated with longer waiting time, while a drop in tonic firing saw rats give up waiting (K. Miyazaki et al., 2011), which can be explained by the framework. On the other hand, these studies also showed results that are at odds with the framework, namely the lack of a 5-HT punishment prediction error during trials in which expected rewards were not delivered. Further, no negative correlation between DA and 5-HT as might be expected for the opposing role the neuromodulators are suspected to play was evidenced.

The involvement of 5-HT signalling in waiting for rewards was further corroborated in studies that used optogenetics[8], which provides an elegant way to control neuronal firing with very high precision and speed (Fenno, Yizhar, & Deisseroth, 2011). Specifically, Miyazaki et al. (2014) modified 5-HT DR neurons to be sensitive to blue light and used microdialysis to measure 5-HT efflux in the MPFC during a delayed reward task. They found that 5-HT neuron activation increased waiting behaviour for a delayed reward, especially after some seconds of waiting, probably when the mouse was deliberating about whether to continue to wait. They further demonstrated that this effect was neither due to a general inhibition effect, nor was the waiting behaviour a result of a potential reinforcing effect of the optogenetic stimulation. This latter finding was replicated by an optogenetic study conducted by Fonseca et al. (2015) who used a similar waiting task in which the animal first had to nose poke at a waiting port and wait until a tone was played after a variable delay before they could move to another port where the reward could be obtained. Fonseca et al. confirmed that light induced activation of DR neurons increased the waiting time for a reward but also reported, in contrast to Miyazaki et al. (2014), that the stimulation increased movement time. To clarify whether this is the result of a pleasant

[8] Briefly, genes that encode for ion channels that are sensitive to light such as microbial opsins are added to DNA segments that are only accessed by a neuron type of interest (i.e. 5-HT neurons), for example via a viral vector or by breeding transgenic animals. The prerequisite is that the specific DNA segments are known. Those ion channels are sensitive to light with a certain wave length (i.e. blue light, yellow light, etc.) and either transport cations or anions into the neuron. For example, channelrhodopsins react to blue light and conduct cations resulting in depolarization of the neuron, inducing an action potential. On the other hand, halorhodopsins react to yellow light and conduct anions into the neuron resulting in hyperpolarization.

or aversive experience during the neuronal stimulation, they tested the influence of photostimulation on choice behaviour when rewards were only delivered in a probabilistic manner. They found that choices were only driven by the high/low probability of receiving a reward, independent of photostimulation. Fonseca et al. (2015) argue that this slowing in movement may be explained by methodological differences, such as the exact neuronal population of the DR that was stimulated. The authors concluded that 5-HT signalling indeed promotes patience, independent of potential reinforcing or aversive stimulation effects and that photostimulation affects behaviour on a very short timescale, which is not predicted by Cools et al.'s framework (2011), implying that slowly changing tonic DA/5-HT levels may induce behavioural changes. While these studies lend overall support for 5-HT activity in intertemporal choices in agreement with the framework, some elements have been challenged, such as the lack of a negative correlation between DA and 5-HT efflux and the fact that 5-HT does not signal a punishment prediction error when rewards are omitted, but rather that phasic 5-HT activity (short timescale) promotes waiting for delayed rewards.

5.5.2. Evidence for 5-HT in reward and punishment processing

As described in the previous section, 5-HT activity as measured in the DR nucleus is causally related to waiting behaviour. At the same time, it does not have a reinforcing effect. The conclusion that 5-HT signalling is only involved in waiting behaviour has been challenged by Cohen, Amoroso, and Uchida (2015) who also used optogenetics to identify 5-HT neurons in the DR and a Pavlovian conditioning to train mice to expect a reward (water or chocolate milk), a neutral outcome (nothing), or a punishment (an air puff delivered to the face or a bitter tasting solution). All outcome categories were presented in blocks to test both 5-HT activity on a shorter timescale (trial-wise) as well as on a longer timescale (block-wise). Additionally, and as a more direct test of Cools et al.'s framework, Cohen et al. also recorded the activity of DA neurons in the VTA. According to their predictions, the DA neurons should show phasic activity for cues predicting reward and increasing tonic activity during a reward block while 5-HT should not respond with phasic activity to rewards and decreasing tonic activity. The inverted pattern is expected for punishment trials/blocks.

Surprisingly, Cohen et al. (2015) observed a very different pattern. First and foremost, the majority of identified 5-HT neurons in the DR revealed higher tonic activity for reward blocks compared to punishment blocks, which is in stark contrast to the framework's prediction that would have DA neurons show such a firing pattern and 5-HT neurons demonstrate low tonic

activity. Even more striking is the observation that the recordings from the DA neurons in the VTA did not evidence any changes in tonic activity between reward and punishment blocks, apparently not coding the assumed long-term reward expectation. However, most of the 5-HT neurons responded to punishments by increasing their phasic firing rate compared to baseline activity. Moreover, around half of the 5-HT neurons also noted phasic activity when an odour was delivered predicting a reward with this phasic activity that was stronger compared to the reception of a punishment. The authors also found the well-known reward prediction error for unexpectedly delivered rewards by recording the large, phasic activation of the DA neurons. Additionally, tonic 5-HT firing was positively correlated to reward magnitude (1µl vs. 4µl) and with the punishments in a way that the authors showed stronger tonic activation to air puffs compared to the bitter solution, indicating that tonic 5-HT activity is related to reward magnitude and different types of punishments.

Cohen et al.'s (2015) findings are in agreement with another optogenetic study (Liu et al., 2014), which demonstrated that optogenetic activation of DR neurons resulted in reward related motivational behaviour such as open space exploration, self-stimulation and learning in a goal directed manner during an operant brain-machine interface task. The authors noted that the reward signalling from the DR interacted with DA neurons in the VTA and nucleus accumbens and while DA neurons showed the expected transient activity to reward predicting cues, the 5-HT neurons were tonically active from reward prediction onwards (again highlighting the role of 5-HT in waiting behaviour) until delivery and consumption. Moreover, the putative 5-HT neurons co-released glutamate that was important but not necessary during reward learning, leading the authors to conclude that the DR nucleus and its neurons are a major reward processing spot and that 5-HT and glutamate together are important for reward related signalling, especially in the VTA and nucleus accumbens. Although this shared influence between 5-HT and glutamate must be better understood, it is tempting to assume that 5-HT controls the motivational part while glutamate activates DA neurons in the VTA and basal ganglia. In this regard, a dual serotonergic signalling model has been suggested (Fischer, Jocham, & Ullsperger, 2014) that ascribes a motivational role to 5-HT including waiting for a reward while glutamate controls the hedonic part of a reward in addition to reward learning, probably via DA neurons.

All of these studies suggest a complex role for 5-HT in reward and punishment processing and although their results differ in some aspects, for example whether 5-HT simply promotes waiting (Fonseca et al., 2015) or actively tracks reward (Cohen et al., 2015; Li et al., 2016; Liu

et al., 2014), 5-HT is unlikely to exclusively communicate punishment related signals and reward only indirectly via dopamine (Dayan & Huys, 2015).

5.5.3. Serotonin functions via different pathways and interactions with other neurotransmitters

The inconclusive and sometimes contradictory outcomes of 5-HT in reward and punishment processing based on pharmacological interventions have been noted by Faulkner and Deakin (2014) while the heterogeneous projection pathways from the raphe nuclei have been reported upon recently by Garcia-Garcia & Soiza-Reilly (2019) as well as Ren et al. (2018) who have strengthened research efforts to better understand the details of the serotonergic system. An early proposal (Deakin, 2013; Deakin & Graeff, 1991) was based on the assumption that 5-HT is concerned primarily with punishment processing and threat assessment in order to prepare a defence response. The authors outline three serotonergic pathways through which 5-HT mediates different, context-dependent functions. These pathways have been topographically validated by a set of studies that used in situ hybridization, histochemistry, immunohistochemical, and microdissection approaches (Paul & Lowry, 2013). One pathway connects the ventrolateral part of the DR with the ventrolateral periaqueductal grey, which is involved in active defence responses such as fight/flight in the presence of a proximal threat, and indicates that 5-HT functions as an inhibitor of these responses under normal conditions. The second pathway connects the median raphe (MR) with the hippocampus, which has been found to mediate coping in the face of chronically aversive stimuli and resilience, possibly by disrupting the consolidation of negative emotional consequences via inhibitory 5-HT1A receptors. The third pathway is primarily engaged in the processing of cues predicting potential threats ultimately inhibiting behaviour and fostering an anxious state which helps to avoid harmful consequences. The authors implicate reciprocal connections between the DR, the amygdala, as well as direct projections from the frontal cortex to the DR in those behavioural processes.

The existence of multiple pathways originating from the raphe nuclei that subserve different behavioural processes further challenges the assumption that 5-HT projections from the raphe nuclei are homogeneous and lead to the desired behaviours with respect to reward/punishment processing using a global dietary intervention. Furthermore, the question arises whether neuronal recordings from the raphe are sufficient as the outcomes that are measured may be dependent on the subpopulation of DR/MR neurons. These questions have been addressed in a

recent study that used advanced viral-genetic labelling and retrograde tracing with image registration in mice to identify and target specific 5-HT subpopulations in the raphe nuclei and their projection sites (Ren et al., 2018). They found two major complementary serotonergic pathways projecting from the DR to the central amygdala and the OFC, respectively. The authors also assessed the significance of these parallel subsystems regarding behaviour by training mice in a lever-press paradigm to receive a sucrose reward and thereafter, mild electric foot shocks while recording 5-HT neuron activity in the DR, OFC, and central amygdala (CeA). They found that both populations reacted similarly to rewards, while the neurons in the OFC pathway ramped up their activity before reward delivery. In contrast, the CeA and OFC pathway neurons revealed an opposite pattern during punishments. The CeA neurons showed a transient increase in signalling followed by a small dip while the OFC neurons demonstrated a longer-lasting decrease of activity. Furthermore, pharmacological activation of 5-HT neurons in the CeA pathway promoted anxiety-like behaviour and suppressed locomotion in an open field test.

On the other hand, the raphe nuclei also receive inputs from several brain regions, indicating reciprocal regulation of activity. Of note are excitatory connections from the frontal cortex, amygdala and hypothalamus, as well as several inhibitory inputs from the basal ganglia (including the striatum), lateral habenula, VTA, SN, amygdala and hypothalamus (Pollak Dorocic et al., 2014). Furthermore, DR and VTA both receive inputs from the lateral habenula to a similar extent, albeit widely differing inputs from, for example, the striatum. There, DA neurons in the VTA and SN receive far more inputs in relation to the DR, which receives more numerous inputs from the extended amygdala network (Ogawa, Cohen, Hwang, Uchida, & Watabe-Uchida, 2014). Most of their functional significance is not yet clear, however, a direct pathway from the vmPFC to the DR was investigated in the context of chronic social defeat stress as DR (and by extension 5-HT) signalling was suppressed by vmPFC via GABAergic neurons resulting in social avoidance behaviour (Challis & Berton, 2015).

Finally, the mediating effects ascribed to 5-HT neurons seem to be dependent on the regulation of other neurotransmitters. It is well known that raphe nuclei 5-HT neurons strongly project to the SN, VTA, striatum, nucleus accumbens and PFC, all of which contain, or are targeted by DA neurons. Further evidence from microdialysis and electrophysiological studies suggests that 5-HT, mainly via its many receptor types, exerts either a direct (5-HT receptors on DA neurons) or indirect (i.e. 5-HT receptors on GABAergic, glutamatergic neurons) effect on DA signalling (Di Giovanni et al., 2008; Di Matteo et al., 2008). Although Di Giovanni et al. (2008) note on page 54 that ‘the prevalent action of the central serotonergic action on DA neuronal activity is

inhibitory', the exact direction may depend on the receptor type. For example, increased 5-HT levels after fluoxetine administration activates inhibitory 5-HT1B receptors located on GABA neurons, reducing their activity and increasing DA release into the mPFC (Matsumoto et al., 1999; cited in Di Matteo et al., 2008). The precise influence of the 5-HT receptor subtypes remains to be elucidated, however, some attention has been provided to the 5-HT1 and 5-HT2 receptor families, as they populate brain regions implicated in decision-making and reward/punishment processing, such as the midbrain, limbic system, and frontal cortex (Beliveau et al., 2017; Di Giovanni et al., 2008; Hayes & Greenshaw, 2011). Some reports suggest that 5-HT1A, 5-HT1B, 5-HT2A, 5-HT3, and 5-HT4 receptors facilitate DA release and function while 5-HT2C receptors inhibit it with the former list of receptors exerting a positive, and the latter receptors, a negative influence on reward processing (De Deurwaerdere & Di Giovanni, 2017; Esposito et al., 2008; Hayes & Greenshaw, 2011).

In recent times, the neurotransmitter glutamate has also been suspected of working in concert with 5-HT and DA neurons in several brain regions. To start, it has been shown that around 60% of DR neurons co-release glutamate to regulate cortical activity (Ren et al., 2018). Second, the DR also receives direct glutamatergic connections from the PFC and lateral habenula (Pollak Dorocic et al., 2014), controlling 5-HT activity. Liu et al.'s intruiging study (2014) suggests a role for both 5-HT and glutamate in reward processing, with 5-HT potentially promoting motivation and/or patience, and glutamate interacting with DA neurons in the limbic system. In particular, Fischer and Ullsperger (2017) have drawn attention to this, suggesting that neither DA nor 5-HT is sufficient for reward processing and that only 5-HT and glutamate release with the accompanying excitement of DA neurons, induce the rewarding effect. Furthermore, increasing 5-HT signalling may trigger aversive processing. Some preliminary evidence suggests the lateral habenula in aversive processing as 5-HT firing increases activity in this area, which on a behavioural level is associated with avoidance behaviour (Tchenio, Valentinova, & Mameli, 2016).

Overall, the evidence presented questions the efficacy of a global 5-HT intervention on complex behavioural traits such as decision-making given the existing pathways and interactions with other neurotransmitters that must be taken into account. The interaction of the 5-HT and DA systems must be better understood, as the Cools et al.'s framework (2011) most like inaccurately represents the roles of DA and 5-HT in reward and punishment processing. Therefore, our null findings following the tryptophan intervention do not necessarily demonstrate that central 5-HT levels did not change, but that additional parameters relating to

the 5-HT system such as the markers for 5-HT receptor protein (Faulkner et al., 2017; Faulkner et al., 2014) or for dopamine and glutamate, require more attention. Another possibility is that different 5-HT pathways are affected dissimilarly by tryptophan interventions. Faulkner and Deakin (2014) reason, for example, that the MR pathway is more susceptible to ATD, as the MR nucleus pathway is relevant for depressive symptomatology and ATD has been shown to reinstate depression symptoms in remitted patients (Delgado et al., 1990). Hence, the effects of tryptophan interventions on the 5-HT system must be studied further using markers that inform about the other neurotransmitters that are involved, as well as the 5-HT receptor subtypes that may provide insights into identifying the pathways that influence VBDM.

5.1. Evaluation of the genetic approach using *5-HTTLPR*

The *5-HTTLPR* has been of major interest due to its association with psychiatric disorders and the fact that it encodes SERT, which is the primary source of eliminating 5-HT from the synaptic cleft and its role in brain development and plasticity (Persico et al., 2010; Serretti, Calati, Mandelli, & De Ronchi, 2006).

We found a significant gene-behaviour association between *5-HTTLPR* and risk-seeking for losses, demonstrating that S/S individuals more frequently chose a potential higher loss than a certain but smaller one compared to S/L and L/L carriers. This finding is in line with previous research implicating the S-allele in higher anxiety traits, higher negative emotional reactivity, and a potential general tendency to avoid negative outcomes such as monetary losses (Serretti et al., 2006). This was corroborated by additional markers in S-allele carriers such as higher amygdala activity towards negative stimuli, reduced grey matter in the posterior anterior cingulate cortex, and reduced functional connectivity between that region and the amygdala, suggesting reduced inhibitory control from the PFC and hence a compromised pathway (summarized in Canli & Lesch, 2007; Hariri & Holmes, 2006). In our third study (Chapter 4) we sought to build on this intriguing idea of reduced top-down control of the PFC to brain regions relevant for VBDM. We chose the uncinate fasciculus, a white matter bundle that connects the frontal lobe with the anterior temporal lobe (including the amygdala) and the frontostriatal tract that connects the frontal lobe with the ventral striatum. However, we did not ascertain a significant difference between the white matter microstructure and risk-seeking for losses and only a non-linear relationship between *5-HTTLPR* and the diffusion parameters that is not easily reconciled with the underlying biological model we assumed. Accordingly, an additional intermediate phenotype in line with our finding may have substantiated the gene-

behaviour association and provided a step towards a biologically informed hypothesis about how genetic effects impact brain structure and eventually behaviour.

Nevertheless, a general criticism with candidate gene approaches such as ours is that they posit a severe simplification of biological processes while ignoring influences from other genes and the environment. As a result, they can only explain small amounts of variance within complex behavioural phenotypes such as VBDM or, more prominently, psychiatric disorders, and their information content regarding the 5-HT system is by extension, incomplete. A prominent example is the role of *5-HTTLPR* in research on depression, following the initial report by Caspi et al. (2003) that the genotype (G) together with environmental (E) influences (G×E interactions) does predict depression. Despite initial support by other studies that implicated the genotype in other psychiatric disorders (suicidality, PTSD, as well as alcohol and other substance use disorders) and a relation to an increased stress response (c.f. Halldorsdottir & Binder, 2017), meta-analyses have shown that the effect of *5-HTTLPR* may either be minimal or a statistical false positive (Border et al., 2019; Munafo, Durrant, Lewis, & Flint, 2009) resulting from underpowered studies and publication bias (Colhoun, McKeigue, & Smith, 2003; Dick et al., 2015; Duncan & Keller, 2011).

Despite efforts to alleviate the situation by recommending more stringent rules that require tighter guidelines with regards to the selection of genes and their variants, more conservative statistical thresholds for multiple testing, and power tests (Dick et al., 2015), the increased affordability of a powerful alternative, genome-wide gene analyses that do not require *a priori* knowledge about what genes and their variants are most appropriate as candidates for certain phenotypes has opened the field to new impulses, such as genome-wide association studies (GWAS). These offer several advantages over candidate genes, mainly that their findings are more robust and replicable in novel data sets (Duncan, Ostacher, & Ballon, 2019) and can be applied to both disorders and traits whose genetic markers are distributed in an unpredictable manner across the genome. The exact method of how to employ GWAS depends on the researcher but offers ample opportunities to better understand the functioning of the 5-HT system from an intermediate phenotype perspective (such as what genetic variances account for differences in 5-HT signalling efficiency) and behavioural phenotype perspective such as reward/punishment processing in VBDM. A viable method is offered through pathway analysis of genomic data (Ramanan, Shen, Moore, & Saykin, 2012), which can, broadly speaking, be conducted either based on biologically-predefined pathways (candidate pathway analysis) or genome-wide ones (GWPA) that include complete genome data. In the interim, such analyses

have delivered some promising results by linking various genetic functions to psychiatric disorders such as schizophrenia all the while validating their findings with independent data sets (Juraeva et al., 2014). Recently, a 5-HT signalling map was published that reports molecular reactions associated with 5-HT or its receptors (Sahu et al., 2018) that may offer fruitful grounds for further elucidation of 5-HT signalling pathways and whether they contribute to intermediate (i.e. brain level) or behavioural phenotypes such as reward/punishment processing. It should be noted that GWAS are not without their flaws and shortcomings, one of which is that they are only able to indicate gene loci without providing any biological explanation for how these contribute to a given phenotype (Ormel, Hartman, & Snieder, 2019).

In conclusion, although we found a plausible genetic association between *5-HTTLPR* and a behavioural phenotype (risk-seeking for losses), we failed to corroborate this finding in an intermediate phenotype (white matter microstructure), which may be due to the effect being too small to be detected within our sample or a diffusion parameter which was too noisy to detect differences in the fibre bundles. Given the discussed limitations of candidate gene approaches and in light of more complex GWAS-related analyses, it seems premature to exclude an influence of 5-HT on probability discounting for losses, however, further genetic analyses that target the 5-HT system on a broader level should be pursued.

5.2. Limitations

Although we examined the serotonergic system based on strong hypotheses mainly derived from an influential framework (Cools et al., 2011) and included the *5-HTTLPR* genotype as prominent marker of 5-HT functioning, there are some limitations that require addressing. First of all, we tested the predictions of the framework only directly from a 5-HT perspective. As a result, we only implicitly controlled for DA levels, assuming that they would remain constant throughout the experiment without assessing markers in the blood or eyeblink rates that would inform about DA activity. It may be possible, as indicated by animal studies, that 5-HT and DA also perform complementary roles depending on the paradigm being tested, which is not predicted by the framework. Especially for DA, it has been suspected that manipulating tonic DA levels, for example with the DA precursor levodopa, does not automatically induce risk-seeking or impatient choice behaviour, but may be dependent on baseline impulsivity as shown by a recent study from our group (Petzold et al., 2019). In the case of 5-HT, it has been demonstrated that the effect of an acute tryptophan intervention on risky choice was dependent on the availability of the 5-HT1B receptor mRNA in peripheral blood, a marker suspected to

correspond to levels in the brain (Faulkner et al., 2017). Furthermore, a PET study by the same group showed that baseline 5-HT1A receptor availability in the hippocampus was associated with higher temporal discounting rates (Faulkner et al., 2014). Both studies present additional markers of 5-HT functioning that we did not take into account.

A second limitation is the varying design of our tasks in relation to animal tasks. The strongest input to computational models and Cools et al.'s framework (2011) came from animal research that informed us how reward/punishment expectations change over the course of an experiment and how this is signalled by DA and 5-HT. However, the tasks used in animal models differ from those utilized in our studies. A prominent difference that may have an impact on the neuromodulators is that nonhuman animals immediately experience the consequences of their choices, both in temporal discounting and probabilistic choice tasks. In our VBDM and ITeCh task, the consequences were not experienced after each trial and only one choice was realized after the task ended. In particular, this may have impacted performance during the probabilistic choice tasks because no feedback was given after each trial, so participants were not aware of the outcome when they chose the probabilistic offer, which may have prevented the adjustment of their reference point on a trial-by-trial basis hypothesized to be signalled by DA and 5-HT. It may thus be that neurotransmitters are more prominent during decision-making scenarios where outcomes are experienced over trials rather than signalling preferences only.

Summarizing, it feels appropriate to point out again that our well-powered study design and sample size should have enabled us to detect small to medium differences for both the intervention ($f = .11$) and its interaction with *5-HTTLPR* ($f = .12$), given a power of 80% and an error rate of 5%. Therefore, I am confident that the null results reported in this thesis are not due to a lack of power but indeed suggest the absence of a significant effect of 5-HT on VBDM.

5.3. Conclusion and outlook

Due to the careful design of our study, we are confident that our null findings regarding the tryptophan intervention are informative for the research community, an opinion shared by several anonymous reviewers who commented on the published manuscripts (see appendix). In the broader context, our findings suggest a gap between the results from nonhuman animal and human studies that should be addressed in the future. One method for accomplishing this would be to employ a close translational approach in which serotonergic pathways discovered in nonhuman animals that are associated with decision-making are targeted, for example, based

on their receptor population and signalling patterns related to specific tasks. Additionally, we would benefit greatly from a more sophisticated understanding of the interactions of 5-HT with other neurotransmitters such as DA and glutamate, as the current models that involve DA are not supported by the data while models that involve glutamate are currently unavailable for decision-making but developed at present only to explain the effect of selective serotonin reuptake inhibitors in depression (Fischer et al., 2014). Additionally, it appears that measuring choice preference in a rather abstract way as we have done where consequences are not dynamically experienced has indicated that they are not primarily influenced by changes in tonic 5-HT levels. Accordingly, the manner in which dynamic and abstract discounting tasks (probabilistic and temporal) influence choice behaviour, as well as how this is reflected in the brain should be clarified, preferably in one sample. This would also contribute to minimizing the gap between human and nonhuman animal studies. A possible avenue to realise this is to use tasks in which the value (based on reward magnitude and probability/delay) of each option has to be inferred or learned, similarly to nonhuman animal tasks, which would deviate from the concept of choice preference, all the while helping determine in which decision-making contexts 5-HT signalling is most salient. While, to the best of the author's knowledge, studies that experimentally manipulated human 5-HT levels and used such a task in the context of risky choice (for example the balloon analogue risk task) have not, to date been published. Schweighofer et al. (2008) used a dynamic task for intertemporal choices, although with a rather small sample size making it difficult to draw conclusions from its findings.

All in all, this thesis provided limited evidence for the influence of 5-HT on decision-making in four tasks. Contrary to the predictions of Cools et al.'s framework (2011) we did not ascertain any changes in behaviour or in the brain response after manipulating tonic 5-HT levels. On the genetic level, we found a significant association between *5-HTTLPR* and risk-seeking for losses. This finding should be taken with caution because it was only related to one task and could not be corroborated by intermediate phenotype markers such as white matter microstructure. As this contradicts nonhuman animal studies in which 5-HT is a prominent neurotransmitter as measured on the neuronal level, this work highlights a gap between the research branches to be addressed in future studies. At this stage however, manipulating tonic 5-HT levels with an acute tryptophan intervention and measuring discounting rates and loss aversion as we have done may not offer the most feasible method for gaining a greater understanding of 5-HT's role in reward and punishment processing.

As a closing remark, the purpose of this thesis was to advance knowledge relating to how serotonin modulates human decision-making from an economics perspective that could either have delayed or probabilistic outcomes. While the decision-making process itself has been extensively studied in the field of behavioural economics offering important insights into human and nonhuman animal biases in decision-making, a mechanistic model remains elusive. The young field of neuroeconomics was founded in 2005 to close this gap by taking the brain as an additional source of information in order to provide a biological explanation for the decision-making biases. At the time of writing, biological models that can contribute to the complex topics of moral philosophy and integrate interactions between social, emotional, and economic aspects, appear to be some distance off. Nevertheless, the field of neuroeconomics has great potential to extend behavioural economics research, while temporary setbacks and obstacles will only serve to improve our theoretical and experimental knowledge in the long run.

6. References

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7. Appendix

7.1. Supplemental material: Study 1

7.1.1. Recruitment workflow

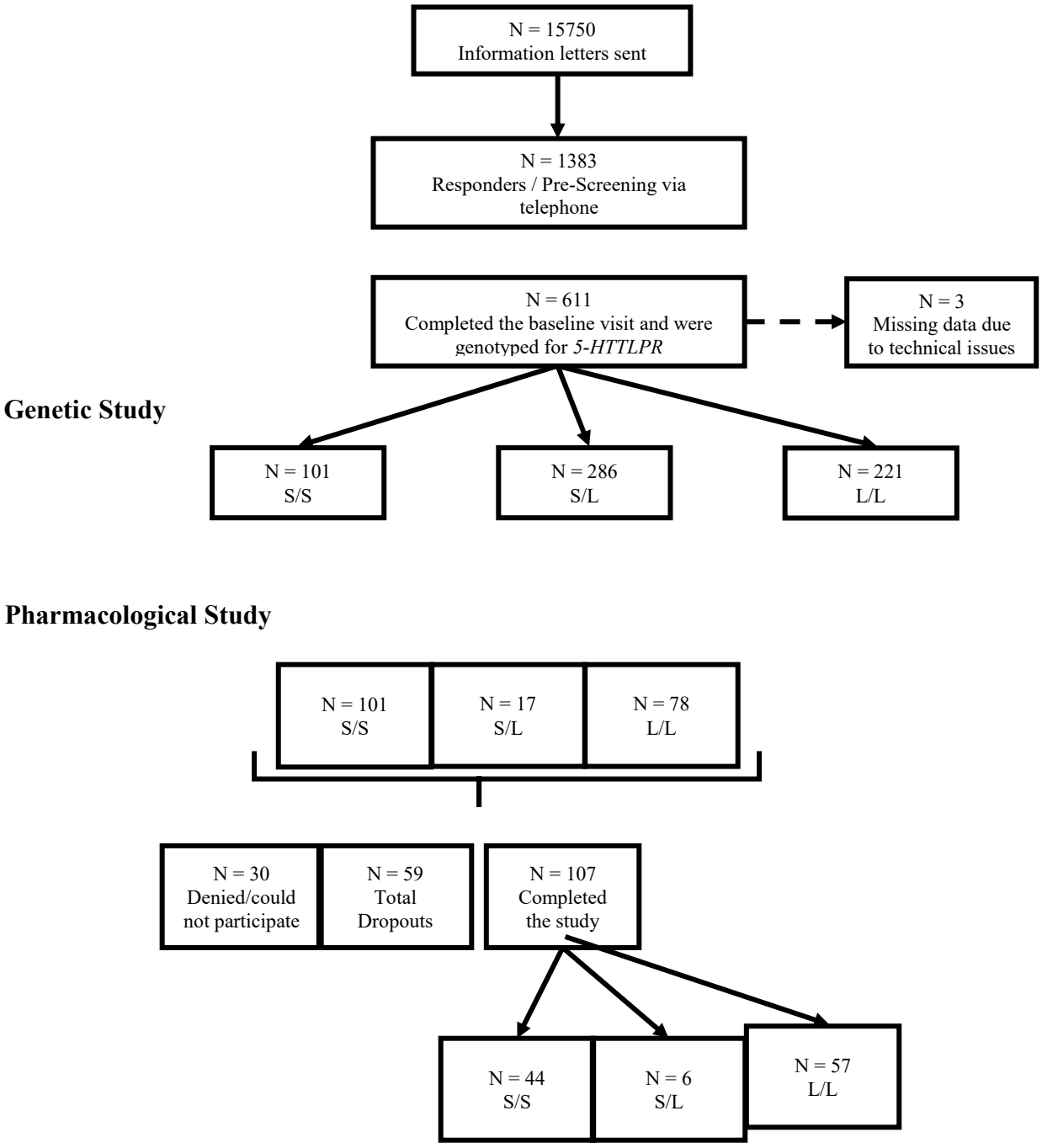

Fig. S1 Recruitment procedure for the genetic and pharmacological study

7.1.2. Baseline visit: Tests & Questionnaires

Behavioural testing of all participants was conducted at computers located in muted booths where they worked on several tasks programmed using the Psychophysics Toolbox (Brainard 1997) in MATLAB (Release 2010a, The MathWorks, Inc., Natick, Massachusetts, United States) and questionnaires (German versions) using a web based application (Milbradt et al. 2007 - 2016) described as follows:

Questionnaires

- Yale Food Addiction Scale (YFAS; Gearhardt et al. 2012)
- Three Factor Eating Questionnaire (TFEQ; Stunkard and Messick 1985)
- Epworth Sleepiness Scale (ESS; Johns 1991)
- Pittsburgh Sleep Quality Index (PSQI; Buysse et al. 1989)
- Short form of the Barratt Impulsiveness Scale (BIS-SF; Patton et al. 1995)
- Arnett Inventory of Sensation Seeking (AISS-D; Arnett 1994)
- NEO-Five Factor Inventory (NEO-FFI; Scandell 2000)
- Fagerstrom Test for Nicotine Dependence (FTND; Heatherton et al. 1991)

Additionally, IQ was measured with the short version of Raven's Standard Progressive Matrices. The MATLAB tasks consisted of the value-based decision-making (VBDM) battery (Pooseh et al. 2017) and a Harvesting task to measure dynamic effort discounting.

7.1.3. Main study visit: General procedure, tests and questionnaires

All participants arrived at three separate days separated at least for a week in order to ensure complete wash-out of the intervention. Participants arrived either at 08.30 a.m. or at 10.30 a.m. at the Neuroimaging Centre (NIC) after following a low-protein diet the day before and having fasted overnight. 1 hour before arrival the amino mixture was prepared by dissolving the powder in 100 ml warm water and mixing it with 200 ml Sprite® soda (after removing most of the carbon dioxide gas from the bottle) to improve taste. In case that the amino acids were not satisfactory dissolved another 100 – 150 ml of tap water was added. Upon arrival, participants gave written informed consent and blood (T0) was sampled via a peripheral venous catheter (Braunüle®), centrifuged for 10 minutes at 4000g, 4°C. 2x1 ml plasma was stored in separate 2 ml Eppendorf capsules in a -81°C fridge. This procedure was repeated three times throughout the session, ~1 hour (T1), ~3 hours (T2) and ~6 hours after drinking the mixture. Afterwards they received either the ATD, ATL or BAL mixture in a randomized, counter-balanced fashion.

Drinking the mixture took 11 minutes on average ± 8 minutes. Only in the first session, they underwent a urine drug screening for amphetamines, methamphetamines, cannabinoids, opiates,

benzodiazepines and cocaine. Additional breathing tests for breath alcohol and carbon monoxide which was performed in all three sessions. Afterwards, participants worked on several questionnaires and tasks related to mood, emotional states and cognitive performance with breaks in between. All tasks and questionnaires (German versions) were used to measure trait, cognitive/executive performance and state aspects of behaviour where traits were measured once per participant and states were measured either once per session or multiple times per session. At noon (either at 12.30 p.m. or 2.30 p.m.) participants went for a 90 minutes MRI scan during which structural (T1, DWI) and functional measures (task-based, resting-state) were obtained. In the afternoon (either at 2 p.m. or 4 p.m.) they completed the PDG/PDL and MGA tasks at the computer. After each session, they received their payment in cash and/or via bank transfer (in total the average win was 196 Euro ± 28) and a snack. Total time for one session was 6.5 hours. During each session they were not allowed to eat, but had access to water ad libitum.

Trait questionnaires measured once per participant in Session 1

1. Buss-Perry Aggression Questionnaire (BPAQ; Buss and Perry 1992)
2. Snaith-Hamilton Pleasure Scale (SHAPS-D; Snaith et al. 1995)
3. State-Trait Anxiety Inventory (STAI-G X2; Spielberger et al. 1970)
4. Melbourne Decision Making Questionnaire (MDMQ; Mann et al. 1997)
5. Demographic information (marital status, education, vocational training, employment status, monthly income, debts)

Cognitive tasks measured once per participant

The cognitive tasks (1-8) were taken from a recently developed battery (Wolff et al. 2016). Furthermore, three more tasks (9-11) were taken from the battery of Lewandowsky et al. (2010):

1. 2-Back (Session 1)
2. Stroop (Session 2)
3. AX-CP (Session 2)
4. Color-shape (Session 2)
5. Category switching (Session 2)
6. Go-nogo (Session 2)
7. Letter memory (Session 3)
8. Number-letter (Session 3)
9. Memory update (Session 3)
10. Operation span (Session 3)
11. Task switching (Session 3)

State questionnaires measured once per session

1. International Physical Activity Questionnaire, pen-paper version (IPAQ; Booth 2000)
2. Perceived Stress Scale (PSS; Cohen et al. 1983)
3. Beck Depression Inventory II (BDI-II; Beck et al. 1996)

State tasks measured once per session

1. Value-based decision-making battery (VBDM; Pooseh et al. 2017)
2. Harvesting task

State questionnaires measured four times per session

1. Visual Analogue Scales for mood (9 Items)
2. State-Trait Anxiety Inventory (STAI-G X1; Spielberger et al. 1970)
3. Karolinska Sleepiness Scale (KSS; Akerstedt and Gillberg 1990)

State tasks measured four times per session

1. Approach-avoidance task (AAT) for food and emotional/neutral pictures

Inspection time (IT; Vickers and Smith 1986) programmed in Presentation© (Version 14.1, www.neurobs.com)

7.1.4. Task correlations across interventions

Table S1 Probability Discounting Gains (logk)

	Depletion	Balanced	Loading
Depletion			
Balanced	.610		
Loading	.379	.445	

Pearson's r

Table S2 Probability Discounting Losses (logk)

	Depletion	Balanced	Loading
Depletion			
Balanced	.449		
Loading	.161	.445	

Pearson's r

Table S3 Mixed Gambles (log λ)

	Depletion	Balanced	Loading
Depletion			
Balanced	.706		
Loading	.619	.582	

Pearson's r

7.1.5. Effect of intervention and *5-HTTLPR* genotype on the VBDM battery parameters, grouped by S-allele: S/S-S/L (n = 50) and L/L (n = 55)

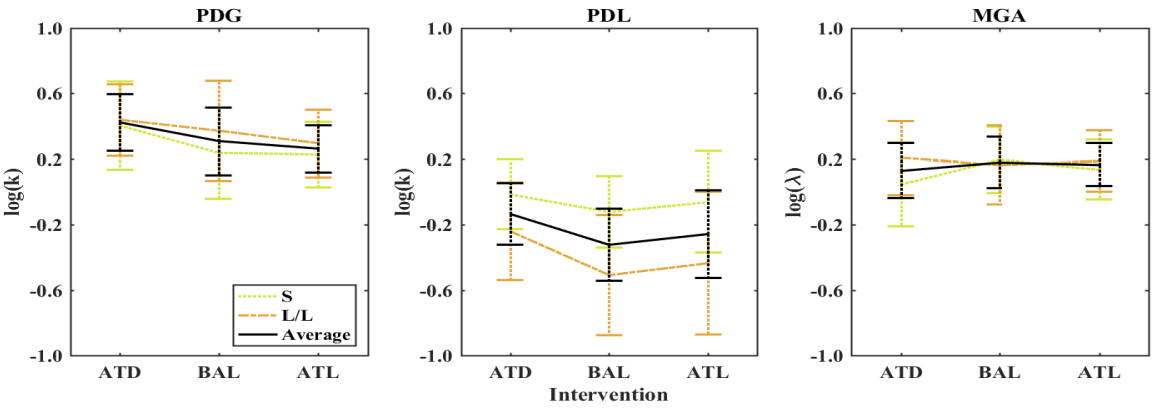

Fig. S2 Effect of functional biallelic *5-HTTLPR* genotype and intervention on three meta-control parameters derived from the VBDM battery tasks. Grouping of genotypes according to the S-allele did not change the results significantly with respect to intervention and intervention x genotype interaction (all $p > .1$). The main effect of genotype did also not significantly change for PDG and MGA (all $p > .5$). However, for PDL there was tendency that the S-allele was associated with higher risk-seeking for losses, which is in line with our finding from the genetic study ($F_{1,103} = 3.637$, $p = .059$, 95% BCa CI [.00521 .65765]. Mean scores and confidence intervals are log scaled and bootstrapped with 10.000 iterations. The error bars indicate 95% bias corrected and accelerated confidence intervals. Abbreviations: PDG: Probability Discounting for Gains, PDL: Probability Discounting for Losses, MGA: Mixed Gambles, ATD: Acute Tryptophan Depletion, BAL: Balanced Condition, ATL: Acute Tryptophan Loading, log(k): Discounting rate k in log scale, log(λ): loss-aversion λ in log scale

7.1.6. References

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7.2. Supplemental material: Study 2

Table S4 Example amount-delay data for a participant with a *k*-value of 0.0183

Delay in Days	Presented amounts in Euro								
0	20.50	20.57	23.83	25.73	30.31	33.63	39.97	62.01	83.82
7	19.96	21.99	23.00	29.21	29.78	35.27	43.25	61.90	83.33
14	20.04	22.79	25.80	26.72	30.20	32.42	40.26	62.29	84.71
21	20.37	21.11	25.84	27.54	29.73	35.18	39.60	61.38	83.25
30	21.04	21.94	23.39	27.16	30.16	35.05	41.68	61.76	81.58
60	20.26	20.55	23.61	27.69	31.13	35.16	39.80	63.38	83.83
90	20.25	22.98	25.03	27.18	29.48	35.24	40.38	61.40	82.48
120	19.60	20.56	25.05	27.48	28.25	34.43	40.41	61.36	84.75
180	20.84	20.25	25.26	29.14	29.25	35.05	39.83	62.44	84.33

The immediate amount is calculated as the subjective value of 30 Euro after 30 days: IM: SV = 30 Euro / 1+0.0183 × 30 days = 19.36 Euro. Each amount level is calculated by the formula: IM + c × (30 Euro – IM), where c was 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0 and 6.0. The nine amounts for this participant were consequently: 20.43, 22.02, 24.68, 27.34, 30.00, 35.32, 40.64, 61.92, and 83.19. All nine delays (0, 7, 14, 21, 30, 60, 90, 120, 180 days) were paired with these nine amounts, resulting in 9×9 = 81 delayed offers. To avoid that the participant always saw the same amounts for each delay, Gaussian random noise with a mean of zero and a standard deviation of 1 was added to each amount, resulting in 81 unique amounts. Offered amounts in red should be rejected, because their subjective value is less than the immediate amount, while offered amounts in green should be favoured over the immediate amount.

Table S5 Brain areas showing activation related to parametric amount magnitude

Region		**BA**	**MNI coordinates**			**Wilk's Λ**	**Cluster size (≥ 60)**
AAL label			x	y	z		
Frontal Med Orb	R	-	0	38	-10	16.2*	144
Cingulum Ant	L	32	-4	40	0	13.8*	
Caudate	R	-	10	10	-4	24.1*	120
Vermis 8		-	0	-66	-28	19.3*	100
Vermis 6		-	-4	-60	-24	18.0*	
Supp Motor Area	L	6	-6	6	66	16.3*	74
Pallidum	L	-	-10	8	-4	20.2*	62

*Peak voxel $p \leq .001$ uncorrected.

Table S6 Brain areas showing activation related to parametric delay length

Region		**BA**	**MNI coordinates**			**Wilk's Λ**	**Cluster size (≥ 60)**
AAL label			x	y	z		
Temporal Mid	L	-	-58	-44	-2	77.2	
Parietal Inf	L	40	-52	-42	40	65.5	
Temporal Mid	R	21	62	-38	-2	65.0	
Parietal Inf	R	-	34	-50	44	58.2	
Temporal Inf	R	-	50	-50	-14	56.3	
Angular	L	-	-40	-66	42	55.7	27352†
Cingulum Mid	R	31	14	-48	36	54.5	
Precuneus	L	7	-8	-62	44	51.6	
Cingulum Post	L	-	-4	-38	32	33.0	
Precuneus	R	-	8	-60	38	32.8	
Lingual	R	18	24	-86	-14	14.3	
Frontal Mid	R	-	38	4	42	52.7	
Frontal Inf Tri	R	-	46	36	6	49.7	
Frontal Inf Oper	R	9	50	14	30	41.8	
Frontal Inf Orb	R	47	54	40	-12	37.5	
Pallidum	R	-	16	-2	6	30.1	8056†
Putamen	L	-	-28	-16	-2	26.1	
Caudate	R	-	10	12	-4	24.7	
Caudate	L	-	-8	12	-6	23.2	
Pallidum	L	-	-20	-8	4	20.7	
Frontal Inf Tri	L	-	-48	36	2	34.2	
Frontal Sup	L	-	-24	62	10	34.2	
Frontal Inf Tri	L	-	-48	30	10	30.2	8872†
Frontal Sup Medial	L	-	-12	64	8	30.0	
Insula	L	-	-26	28	2	19.9	
Frontal Inf Orb	L	-	-38	44	-16	17.0	
Cingulum Ant	R	32	16	42	6	19.9	490†
Cingulum Ant	L	32	-6	42	8	15.9	
Cingulum Mid	L	-	0	6	32	27.0	224†
Cingulum Mid	R	-	2	-6	34	16.9	
Frontal Sup Medial	R	8	4	44	46	14.4	222†
Frontal Sup Medial	L	-	-6	40	42	14.0	

†Corrected at cluster-level p(FWE) < .05, cluster-forming p = .001 uncorrected.

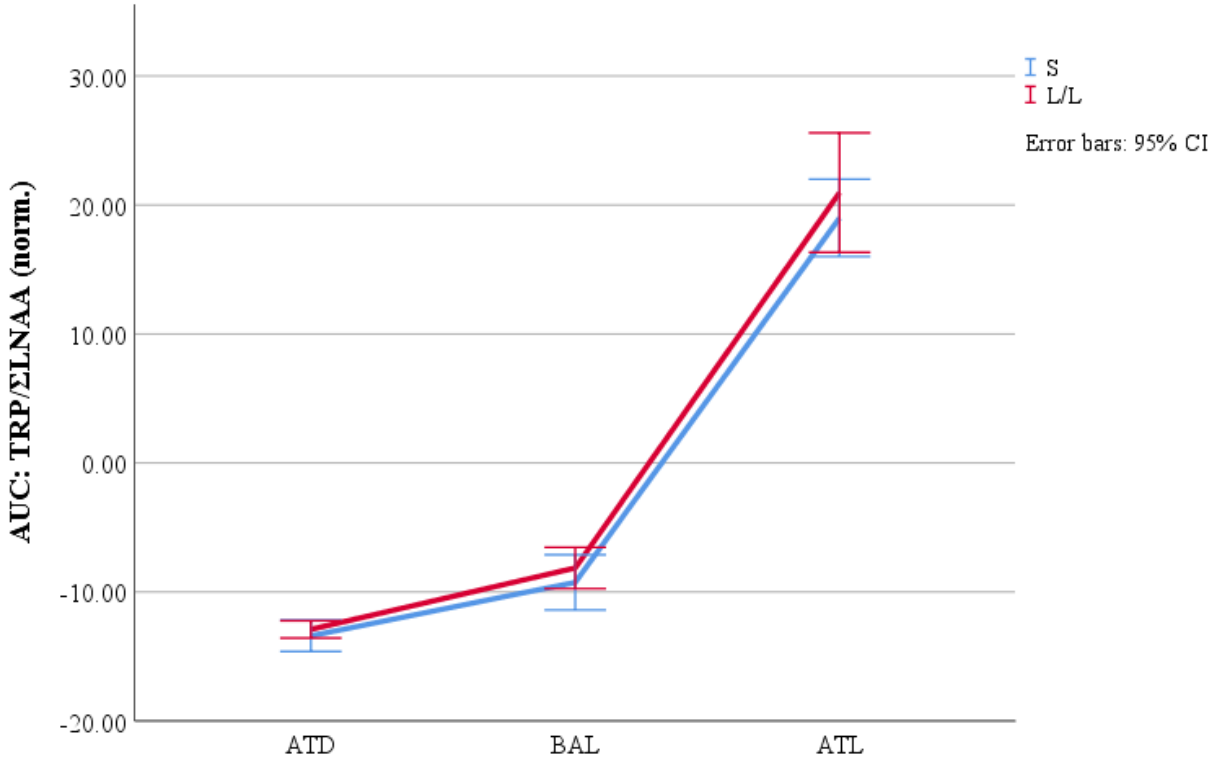

Fig. S3 Effects of intervention and *5-HTTLPR* on the areas under the curve (AUCs) from 80 participants. The AUCs were computed from the four time points were venous blood was taken (T0: before drinking the amino acid mixture, T1: One hour after drinking the mixture, T2: Three hours after drinking the mixture, T3: Six hours after drinking the mixture). We used the ratio of tryptophan (TRP) to the sum of the other large neutral amino acids (LNAA) as parameter of interest and subtracted T0 from each other time point to account for interindividual basis levels. We then computed the AUC for each intervention condition (ATD: acute tryptophan depletion, ATL: acute tryptophan loading, BAL: balanced condition). The AUC of ATD was significantly smaller than BAL and the AUC of ATL was significantly higher than BAL (all $p < .00001$). There was however no significant main effect of *5-HTTLPR* genotype, nor an interaction effect.

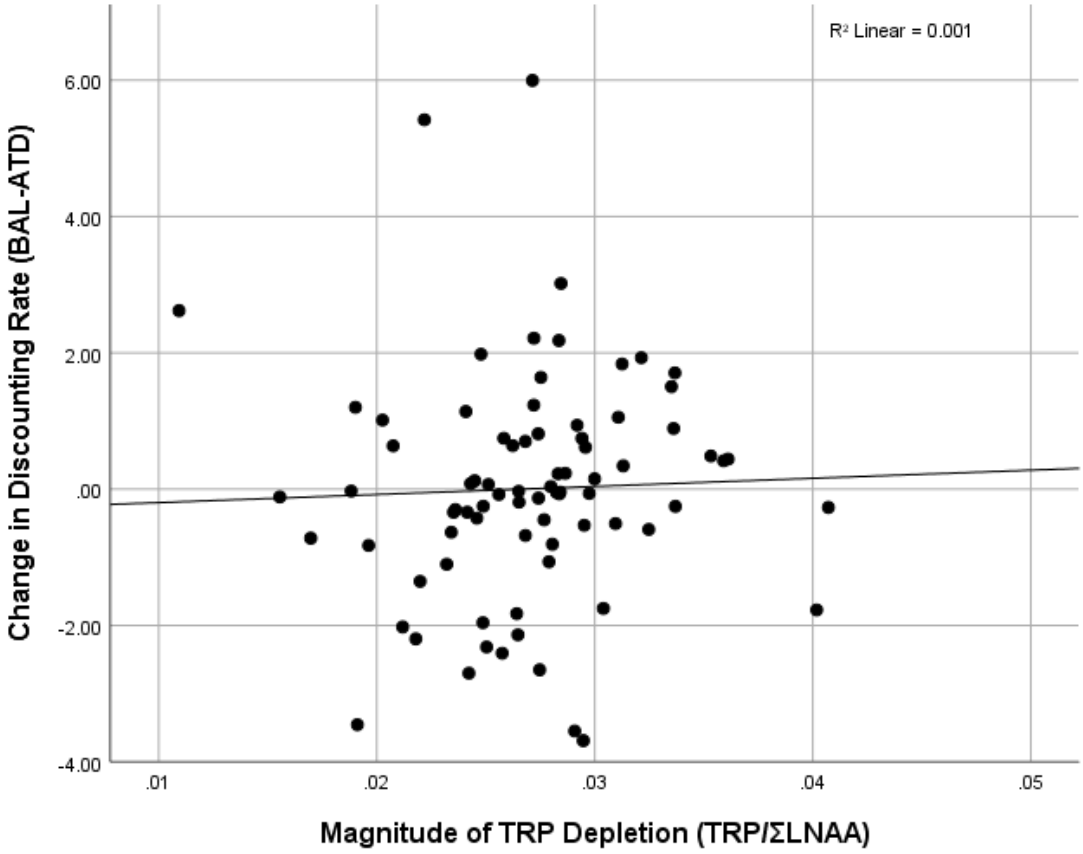

Fig. S4 This plot replicates the analysis of (Crockett et al., 2010) and shows no correlation between changes in discounting rate between the balanced and depletion condition and the magnitude of TRP depletion.

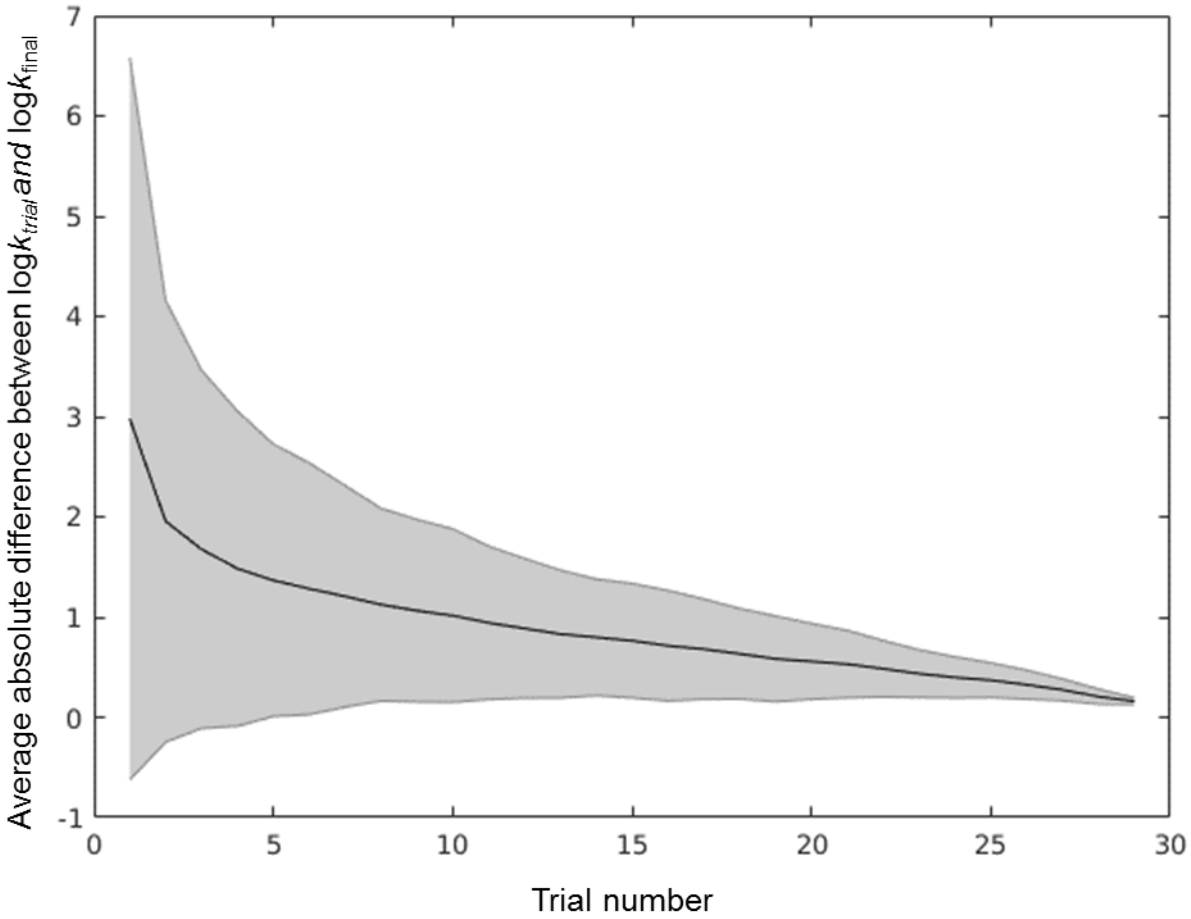

Fig. S5 Convergence plot of the sequential Bayesian trial-by-trial estimation of the discounting rate from the delay discounting (DD) task used in the genetic study. The black line shows the average absolute differences between the estimation at each trial and the final estimation for all participants. The grey area depicts the one standard deviation distance from the average.

7.2.1. Reference

Crockett, M.J., Clark, L., Lieberman, M.D., Tabibnia, G., Robbins, T.W., 2010. Impulsive choice and altruistic punishment are correlated and increase in tandem with serotonin depletion. *Emotion* 10, 855-862.

7.3. Supplemental material: Study 3

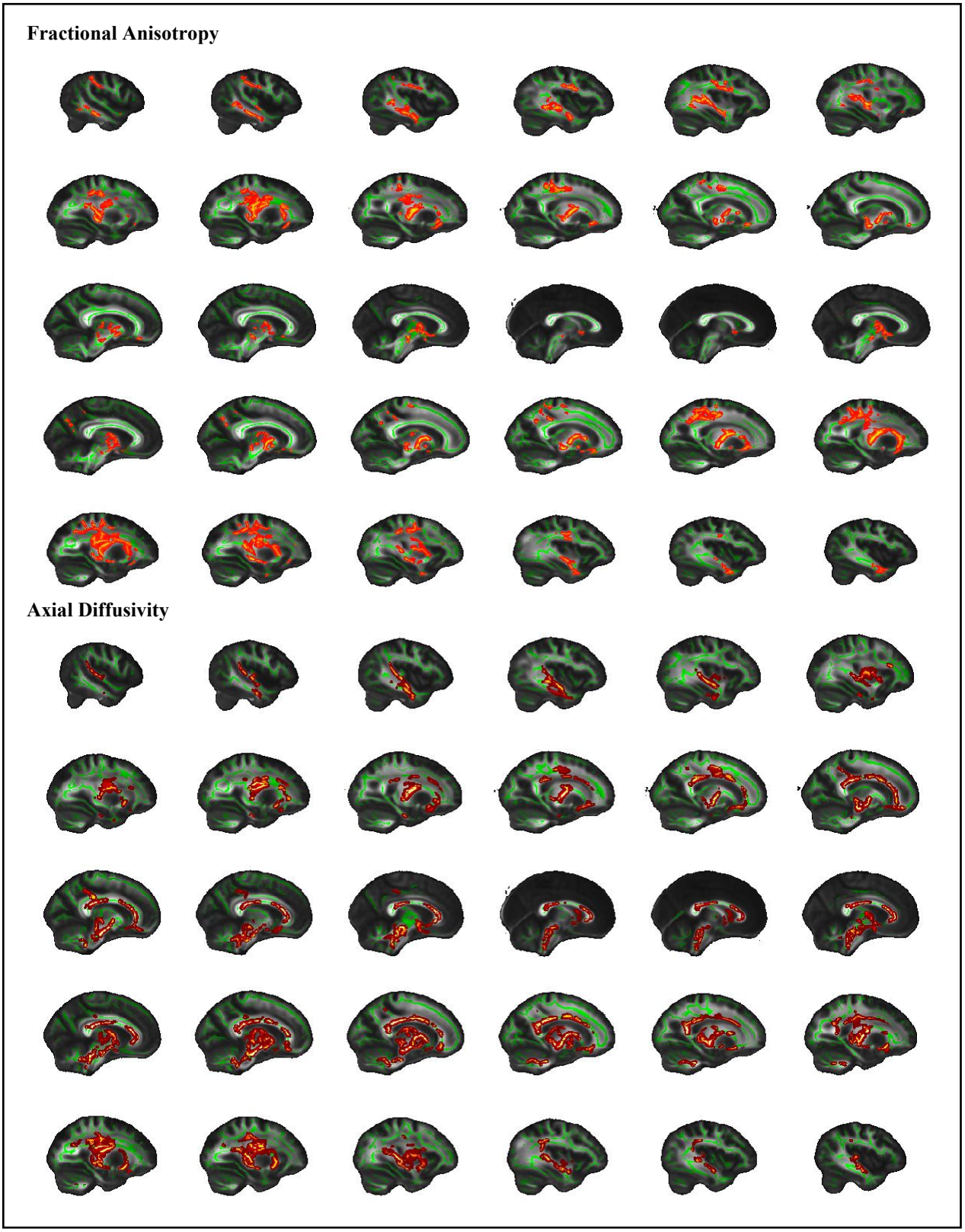

Fig. S6 TBSS main effect of *5-HTTLPR* based on 10.000 permutations for fractional anisotropy and axial diffusivity. All voxels survive a family-wise error correction of $p < .05$.

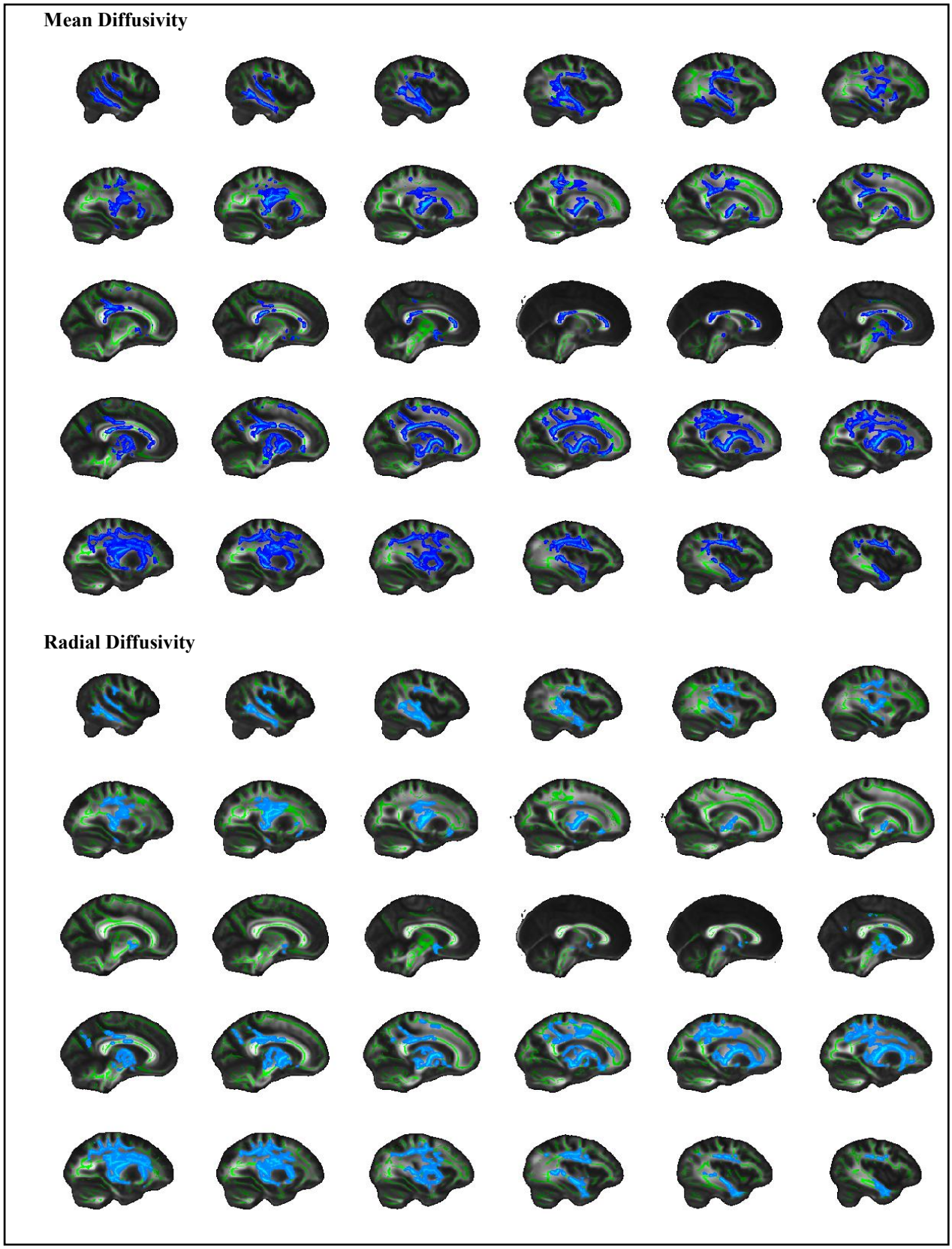

Fig. S7 TBSS main effect of *5-HTTLPR* based on 10.000 permutations for mean diffusivity and radial diffusivity. All voxels survive a family-wise error correction of $p < .05$.

7.4. Reviewer's comments: Study 1

Reviewer #1: This is an excellent manuscript detailing the influence of central serotonin levels and 5HTTLPR genotype on risky decision-making. The authors report that risk-seeking for losses (but not risk-seeking for gains or loss aversion) depended on genotype but not manipulations of central serotonin levels; there was also no genotype*treatment interaction. The authors should be commended on a number of points, including the sample size, the use of both tryptophan depletion and tryptophan loading (as well as sham depletion, of course), and for sampling plasma tryptophan levels at multiple time points between 0-6 hours post-treatment, which is rarely done. I recommend this manuscript for publication, after a number of comments have been tended to.

Major comments

1. On page 3 (page 1, Introduction), the authors discuss how serotonin is considered to be involved in the processing of information pertaining to punishments. 'More specifically, tonic dopamine (DA) levels are assumed to code for the average reward rate, linked to the opportunity costs which states how vigorously actions should be performed to receive rewards and tonic 5-HT encodes the average punishment rate, indicating the costs of action vigour.' This is also discussed at other points throughout the manuscript. While this is considered true by many, others have argued that serotonin is also involved in rewards. For example, Rogers et al (2003) report that acute tryptophan depletion (ATD) alters healthy participant's ability to process rewards. This should be noted and discussed. How do the authors marry the results of Rogers et al with their own? This should be noted in the introduction.

> ***Answer:*** *First, we apologize for the confusion we have caused by not clearly describing the framework. We revised the description of the model on the first page of the Introduction section. It is correct that in this framework 5-HT codes for the average punishment rate (and dopamine for the average reward rate). However, the idea of the framework is that both, the average reward rate and the average punishment rate TOGETHER determine the overall average outcome (difference of average reward and average punishment) and hence 5-HT also effects the processing of rewards. To make this pivotal point clearer we revised the description of the model in the Introduction*

section (p 3, paragraph 1,2). Furthermore, we included the findings from Rogers et al. (2003) into our introduction and relate them to the framework. However, we believe that it is easiest for the reader, if we relate our results to Rogers et al. (2003) in the discussion section.

2. Page 3, paragraph 4, introduction: The first sentence is not clear enough. For example, which computational approach exactly? This should be spelled out more. Further, the second sentence is not clear enough either; 'In line with the predictions of the model' - what model exactly? I know what you are trying to say but things should be made easier for the reader.

Answer: *We acknowledge that the terminology was not constant throughout the manuscript, which may be confusing for the reader. Therefore, we now use a coherent term to refer to the framework by Cools et al. (2011) and described shortly the predictions of the framework to make reading easier (p 4, paragraph 1).*

3. Page 4, line 10; the results of Chamberlain et al (2006) should be discussed in light of evidence indicating that a single, acute dose of citalopram actually decreases central serotonin levels due to actions at inhibitory 5-HT1A receptors in the raphe nuclei - the results of Selvaraj et al (2012) and of Blier (1998) should be referenced here in support. This means that the effects of citalopram on 'loss sensitivity' as it is described may be due to a sudden decrease in serotonin, although it is impossible to know for sure. You can then use this difficulty in interpreting the serotonergic effects of single, acute citalopram as a motivation for administering ATD/ATL instead, which or sure have effects on the serotonin system that are easier to clarify.

Answer: *We would like to thank the referee for highlighting this important issue! We followed his/her advice and used the uncertainty regarding the mechanism of SSRIs on 5-HT levels to motivate the use for our tryptophan intervention (p 4, paragraph 2)*

4. On page 4 you say 'In this way, we are able to cover multiple facets of decision-making behaviour in a larger sample, thus avoiding potential methodological issues' - a larger sample than what? What were the sample sizes of the studies you have cited above, if you are referring to these?

Answer: *We agree with the referee that this statement lacks precision, therefore we state that our sample size was two to three times higher than in previous studies and referenced five example studies (p 4, paragraph 2).*

5. Page 5, line 7; The results regarding the IGT are not clear enough - for the uninitiated, 'disadvantageous decks' will not mean so much. Perhaps provide a short description of the task first. Also, define 'better performance'.

Answer: *We followed the referee's comment and included a brief description of the task and explained the terms dis-/advantageous decks (p 5, paragraph 2).*

6. Page 5, line 26; as in my major point 2 above, the term 'the computational framework' is not defined well enough - please describe this in a little more detail so the reader does not have to think back to what you said above.

Answer: *We unified the term so that it refers directly to the framework by Cools et al. (2011) and pointed out what exactly the prediction of the model was in the respective context (p 5, paragraph 2).*

7. Page 5, line 35 - you say that Ernst et al (2014) reported the 'opposite direction in anxious adolescents with L/L genotype' - does this mean the same direction in those with the S/S genotype, or no effect in this group? This needs just a few words for clarification.

Answer: *We agree that this statement is not easy to follow and changed it in a way that the L/L genotype group of anxious adolescents showed reduced loss-aversion with no effect in the anxious S/S genotype group (p 5, paragraph 2).*

8. Page 6, line 57; Please state how many participants had all three missing data points - and subtract from the 611 completing subjects.

Answer: *We followed the referee's comment and described the missing data in relation to the 611 completing subjects (p 7, paragraph 2).*

9. Page 7, line 10; Please state the criteria by which these participants were deemed eligible.

Answer: *We agree that we did not state the inclusion criteria exactly for the pharmacological study and therefore we included our recruitment rational (p 7, paragraph 3).*

10. Page 7, line 16; Is this therefore a within-subjects design? If so, please state this.

Answer: *We follow the referee's comment and included a brief statement that the design is indeed within-subject (p 8, paragraph 1).*

11. Page 9, line 47; why are there three separate rm-ANOVAs? One for each outcome from each task? Or one for ATD, one for ATL, and one for BAL? I presume it is the former with ATD, ATL and BAL as one categorical factor, but this needs to be calrified. Same for the models described on line 50.

Answer: *We apologize for the confusion and clarified that the rm-ANOVAs were done separately for each task (p 10, paragraph 5).*

12. Page 9 line 56. This paragraph should be moved to the second in the 'Statistical Analysis' section - it makes sense to test the efficacy of the treatment before anything else.

Answer: *We agree with the referee and moved the blood plasma analysis paragraph before the VBDM data analyses of the pharmacological study (p 10, paragraph 4).*

13. Page 12, line 38; In this paragraph, were the effects of ATD and ATL specifically compared? I apologise for this, I simply am not sure.

Answer: *No, in contrast to the first paragraph where we tested a planned linear contrast of ATL > BAL > ATD (1 0 -1) and vice versa with a one-sample t-test, we explored in this paragraph whether the three outcomes of the VBDM tasks differed between any of the three conditions (ATD/BAL/ATL) with an F-test (p 12, paragraph 2).*

14. Page 13, line 1; Again, the computational model in Cools et al (2011) should be briefly described.

Answer: *We followed the recommendation and included a brief description of the framework (p 12, paragraph 4).*

15. Page 13, line 34; please could the authors speculate as to why the results of Talbot et al (2006) differ from those of Rogers et al (1999) - differences between participant groups for example?

Answer: *We agree with the referee that these differences merit more attention and included an extra paragraph to discuss this matter in the context of inter-individual differences related to 5-HT functioning that may have affected the effect of the acute tryptophan interventions. As a result, and to maintain readability we changed the structure of the discussion by moving our description of the findings from Anderson et al.*

(2003) to the beginning of the discussion about the pharmacological study (p 12, paragraph 5 and p 13, paragraph 2).

16. Page 13, line 52; The authors discuss Rogers et al (2003), but not those of Faulkner et al (2016) which show that the effects of ATD on the processing of information pertaining to rewards in a risky decision-making task depend on individual differences in levels of 5-HT1B receptor mRNA in the periphery. The authors should report Faulkner et al (2016), and potentially use it as an argument as to why there was no effect of ATD (just as there was no effect in the whole group in Faulkner et al, 2016). Individual differences always need to be considered, which may be one reason for the discrepancy in results between Talbot et al (2006) and Rogers et al (1999). In the same argument, the authors should refer to the results of Faulkner et al (2014) which show that individual differences in 5-HT1A availability are related to processing of information pertaining to rewards on the same risky decision-making task as that used in Rogers et al (2003) and Faulkner et al (2016).

Answer: *The referee made an excellent point in highlighting the importance of biomarkers such as peripheral 5-HT1B mRNA availability for explaining inter- individual differences in the effect of acute tryptophan interventions. As stated in the previous comment, we used an extra paragraph to discuss the finding of Faulkner et al. (2016) as a potential reason why Rogers et al. (1999) and Talbot et al. (2006) had opposite results and added this mechanism as a potential reason why we did not observe a net effect of our intervention (p 13, paragraph 2).*

Minor comments

1. Page 3, paragraph 3, introduction: 'mixtures that lacks tryptophan' should be changed to 'mixtures that lack tryptophan'.

Answer: *We changed the word accordingly (p 3, paragraph 3).*

2. Page 5, line 7; 'IGT' should be defined as the Iowa Gambling Task before the acronym appears.

Answer: *We included the full task name before the acronym accordingly (p 5, paragraph 2)*

3. Page 6, line 37; 'visus' should be defined.

Answer: *We described 'visus' now more precisely as 'visual acuity' (p 7, paragraph 1)*

4. Page 8, line 59; I believe this blood is collected for assay of plasma tryptophan levels - if this is correct, please state this.

Answer: *Following the referee's recommendation, we state now at the beginning of the paragraph that we collect blood to assay plasma tryptophan and LNAA levels (p 8, paragraph 3).*

5. Page 8, line 11; please redefine PDG, PDL and MGA

Answer: *In order to avoid confusion about the acronyms we defined them in the first sentence of the paragraph (p 9, paragraph 1).*

Reviewer #2: The exact role of 5-HT in value based decision making has been a central issue in recent development of cognitive psychology and decision neuroscience. However, data from both animals and humans in the past decades were inconclusive. The authors were ambitious in that they were focusing on both the genetic (5-HTTLPR) and transient level of 5-HT on subjects' decision bias using a novel task battery and estimation method (Pooseh et al., 2017). They found that S/S individuals are more risk seeking in the loss domain of the decision task and the acute manipulation of 5-HT showed no effect. Their results added another piece of intriguing data to the already hotly debated role of 5-HT. While I in general like the approach they took and appreciate the large sample size, I think the paper can improve significantly by considering the following points:

1. According to the expected utility/value theory, what the authors really estimated was not risk attitude per se, but rather probability distortion factor. Such a factor does influence people's risk attitude but it does so in conjunction with the marginally decaying power parameter. This is especially the case when the mixed game is concerned since in this case the probability is 50-50 and previous literature indicates less/least probability distortion here. I would like to see the authors augment their model by including the power parameter in their utility function (instead of the linear one that's currently presented) and see whether this would change their results.

Answer: *We acknowledge the concerns of the referee about different usages of the terms regarding risky behavior in the literature. To the best of our knowledge, according to the*

prospect theory (Kahneman and Tversky 1979; Tversky and Kahneman 1992), probability distortion is related to the undervaluation of large probabilities and overvaluation of small ones. Therefore, we deliberately chose probabilities from a range, $1/5 \leq p \leq 2/3$, that is linear in distortion and not close to zero and one, to avoid this effect. On the other hand, estimating discounting rates (temporal and probability) by fitting two-alternative forced choices to the simple hyperbolic value function is a common practice (Green and Myerson 2004; Madden et al. 2009; Myerson et al. 2001; Petry 2012 and references therein). To this end many studies have used curve fitting techniques that need many trials compared to our novel Bayesian method which gives reliable estimates using less trials (Pooseh et al. 2017). We believe that adding more parameters to our model is feasible but decreases the power having a number of 30 (40) data points in this study. To make sure, we conducted simulations, as we did in Pooseh et al. (2017), using the hyperboloid value function, $V=A/(1+k\theta)^s$ (Green and Myerson 2013), which integrates the power weighting of amounts and the odds against winning into the simple hyperbolic function. We sampled 1000 tuple of (k, β, s) and simulated data sets with these preset parameter values by running our tasks on them to get the corresponding estimations. So for any (k_i, β_i, s_i), we get a (k'_i, β'_i, s'_i). The squared correlations, r^2, between the preset and estimated parameters are measures of the reliability of the parameter estimation. For the case of the simple models with two parameters as we have reported in Pooseh et al. (2017), the values of r^2 were 0.96 and 0.90 for k and β respectively. For the augmented model, the reliability coefficients are 0.85, 0.00 and 0.48 for k, β and s respectively. This, as we expected, demonstrates poor reliability for s and completely unreliable estimates for β. We therefore conclude that adding the s parameter is not a useful approach for our task and data.

2. The parameter estimation using a Bayesian approach is an interesting venue but the prior for these parameters should be fully described and sufficiently justified. 30 or 40 trials might not be enough to wash out the prior influence and have a good estimation if they used a very narrow prior. Also, the fact that every subsequent trial offers were presented exactly at the indifference points seems too easy to be influenced by a few "slip of the brain" bad decision trials. Such an approach might increase the efficacy of parameter estimation but intuitively at the cost of great susceptibility to a few "irrational trials". I'd like to hear authors opinion on this issue.

Answer: *We thank the reviewer for raising these issues. The priors on the parameters are normal distributions with large variances and means in the middle of the range of the respected parameter. For instance, we assumed that -10 ≤ k ≤ 10 and set the mean of the prior 0 with a variance of 30. These priors are almost flat and do cause the least bias in the estimation procedure. Following the referee's recommendation, we added this important information to the manuscript (p 9). Presenting offers from the indifference point yields the most informative observations theoretically and any* "slip of the brain" *would not harm that much because both options have the same value. For instance, if at any trial the probability of choosing the risky option is 0.95 and a subject chooses the certain option, this would be problematic since the behavior is the opposite of the expected one. Nevertheless, at the indifference point, where the chances of taking either of the options in 50%, any choice would be very close to what the model expects. Furthermore, we present randomly chosen offers every fourth trial in our procedure to avoid reverse engineering the pattern by subjects and any unknown effect of staying close to the indifference point in successive trials. At every trial, we present offers from the indifference point based on the current estimates that are far from the true parameters of the subjects. Nevertheless, the posterior distributions on the parameters change drastically during the initial trials and after reaching a stable point, where we believe the estimates are good enough, the posterior distributions do only change slightly. Regarding the fact that we have recently published all technical and mathematical details of our algorithm in Pooseh et al. (2017), repeating them in this paper seemed redundant to us.*

3. In the introduction, the authors mentioned that they expected to see S/S individuals to be less risk seeking but the results were the opposite. This in turn makes me wonder whether that's because their model is not sufficiently complex enough to really capture the risk sensitivity parameter.

Answer: *The referee raises an interesting point, which was one of our concerns as well. As we mentioned in the discussion, our battery could discriminate a group of healthy controls from alcohol dependent patients (Bernhardt et al. 2017). Furthermore, it has been shown by simulation (Pooseh et al. 2017) that our algorithm gives reliable estimates of its model parameters and at the same time reveals comparable results to some well-established models (references in the response to question 1). Making the model more complex on the other hand results in unreliable estimations as we pointed out earlier*

4. The pharmacological study only had around 100 subjects (S/L individuals less than 10), I would encourage the authors to divide their genotype into two groups and have a pharmacological manipulation (ATD/BAL/ATL) by genotype plot and show whether there is any main or interaction effect in each task.

Answer: *We followed the referee's advice and repeated the analyses with only two genotype groups. The results however did not change if we assigned the S/L groups to either the S/S or L/L genotype or exclude them entirely from the analysis as can be seen from the table below. We included this statement into the Results section.*

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7.5. Reviewer's comments: Study 2

Reviewer #1: This article from an excellent team details an examination of the role of serotonin function in delay discounting. In examining the influence of both serotonin transporter and manipulation of central serotonin levels on both behaviour and the associated brain function, this manuscript proves to be both interesting and (I believe) important to the field. It uses excellent, scientifically-sound methodology (particularly with regards to the fMRI methods) and statistical analyses.

Answer: *We wish to full-heartedly thank the reviewer for his/her positive assessment of our study, especially with respect to the methods!*

Major points:

1. On page 4 (starting on line 42), you are correct when you state that Schweighofer et al report that compared to depletion, tryptophan loading increased discounting. However, please also include the finding that Schweighofer report that compared to sham (i.e. control), tryptophan loading decreased the discount parameter, but that depletion had no effect compared to sham. I believe that this latter point supports your findings, and so is important to include.

Answer: *We thank the reviewer for pointing out to also include the relationship between the sham and loading condition. We changed the sentence on page 4 as follows:*

"The other study manipulated 5-HT levels using an acute tryptophan intervention with a depletion, a sham and a loading condition. They reported that the depletion condition increased temporal discounting compared to the loading and sham condition (Schweighofer et al., 2008), but the loading condition had no effect compared to the sham condition".

Importantly, the significant finding was indeed driven by the depletion and not by the loading condition, which was wrongly represented in the Figure 2 legend. Consequently, Schweighofer et al. published an erratum (https://www.jneurosci.org/content/28/21/5619) with the correct figure legend.

2. The small number of trials (30) should be noted in the discussion as a (potential) limitation. Many studies of intertemporal choice examine discounting over hundreds of trials (e.g. Pine et al. 2007). Please discuss the fact that this (i.e. low statistical power)

may be a reason as to why you did not find a relationship between the transporter and discounting. Consider the few recent reports that statistical power is based not just on the number of participants, but also on the number of trials.

Answer: *We agree with the reviewer that, in general, more trials should increase the reliability of the discounting parameter and that Pine et al. (2009) have much more trials (220) compared to our study. However, it has also been suggested that temporal discounting is a robust construct that can be accurately measured after only 5 trials (Koffarnus and Bickel, 2014). Therefore, we believe that 30 trials should be sufficient. Furthermore, in our task we used an adaptive Bayesian algorithm, which presents offers close to the indifference point at each trial updating the parameter estimates based on all previous choices, which requires much fewer trials to yield robust results. Simulations described in Pooseh et al. (2018) clearly show in Fig. 2 that only 10 trials are needed to estimate k, given higher choice consistency (beta) values. Conversely, with lower beta values, the estimation benefits from 30 trials. To show the robustness of the k parameter estimation, we include a graph with the average trial-by-trial estimation and convergence across all 589 participants in the supplemental material Figure S3. We discuss it on page 30: "One may argue that the number of trials used to estimate the discounting rate in our study was rather small and might result in low reliability of the discounting parameter, which might finally reduce statistical power of our study. Our data indicate, however, that the parameter estimation showed strong convergence after already 25 trials and are robust until the last trial (cf. supplemental material Figure S3). Therefore, we believe that due to the adaptive Bayesian algorithm used in this task, we have reliably estimated discounting rates after 30 trials".*

3. Please note why the 'amount of amino acids… is less than what is used in other studies', as described on page 11.

Answer: *We thank the reviewer for pointing this out and added the following explanation on page 12: "The main advantage of this mixture is that it reduces the risk of nausea that has been reported earlier (Booij et al., 2005, Cools et al., 2005, Schmitt et al., 2000) while still effectively changing 5-HT levels (Biskup et al., 2012, Dingerkus et al., 2012)".*

4. While your statistical analyses seem perfect, please consider also including simple Bayes Factors along with frequentist p values. Such an inclusion would allow you to more firmly conclude that there is no role for serotonin (at least as you have measured it) on delay discounting, because this is what a Bayes Factor of >0.3333 allows. Including only frequentist statistics only allows you to conclude, for example, that 'we failed to find evidence for a role for serotonin in delay discounting because $p > 0.05$'. This is a very simple task and can be done in SPSS v25, or JASP if needed.

   Answer: *We thank the reviewer for this great suggestion, which indeed helps to highlight the claims we make in this manuscript! We conducted Bayesian statistics with JASP (reported on pages 10/11 and 16) and included the resulting Bayes Factors into the results section, pages 20 and 23).*

5. On page 25 (discussion section) you state that 'Crockett et al (2010) reported that the extent of the pharmacokinetic effect of ATD… was correlated with the increase in delay discounting' Did you find anything similar in your data? Either way, please report this and discuss briefly.

   Answer: *We thank the reviewer for this comment and point out that we were also curious and looked for such a correlation in our data and did report it in our discussion (now on page 26) with the following sentence: "Using a comparable experimental design, we also could not observe a main effect of our interventions nor an effect proportional to the pharmacokinetic parameters (supplemental material Figure S3). Therefore, our results might indicate that discounting behaviour during tasks that investigate individual time preferences is not substantially modulated by 5-HT neurotransmission". We apologize if this was not clear enough. Nevertheless, we feel encouraged to provide a plot of our data in the supplement as Figure S2.*

6. Consideration needs to be given to pertinent work from Jon Roiser's lab on this topic. Specifically, you should discuss his work showing a role for the 5-HT1A receptor in intertemporal choice (Faulkner, Selvaraj, Pine, Howes and Roiser., 2014; Psychopharmacology) and discuss how this fits with your current results. Further, his lab has also shown how individual differences in 5-HT receptor types can mediate the effect of tryptophan depletion on decision-making (Faulkner, Mancinelli, Lockwood,

Matarin, Dolan, Wood, Dayan and Roiser., 2016; International Journal of Neuropsychopharmacology) – such results may have bearing on your own. Indeed, they also report that expression of the serotonin transporter gene does not influence the effect of TD on decision-making, which supports your own results. Please discuss this study also, taking into consideration how such findings may partially explain your own.

Answer: *We thank the reviewer for raising this point. Indeed, Faulkner et al. (2014; 2016) are interesting findings, which we included now in our discussion on page 28: "It should be noted that we were not able to account for individual differences in the make-up of the 5-HT system, namely the role of 5-HT receptors, which have been shown both to play a role in intertemporal choice and to interact with tryptophan interventions. For example, Faulkner et al. (2014) used positron emission tomography (PET) and provided preliminary evidence that increased temporal discounting was associated with reduced 5-HT1A receptor availability in the left hippocampus, probably related to prospective memory ability. A second study by the same group (Faulkner et al., 2017) showed that peripheral measured baseline levels of 5-HT1B receptor mRNA (a proxy for central 5-HT1B receptor mRNA levels) modulated the effect of ATD in a risky choice task. Specifically, 5-HT level changes in the depletion (compared to the sham) condition and higher 5-HT1B receptor mRNA levels were associated with increased gambling and increased sensitivity to larger wins. Very interesting for us is that they did not find an association with serotonin transporter mRNA levels, which suggests that the transporter is indeed not strongly involved in temporal and risky choice and highlights the importance of taking other markers of the 5-HT system (i.e. 5-HT1A, 5-HT1B receptors) into account".*

Minor Points:

1. Please provide a reference for the line beginning 'Together, the neuromodulators 5-HT and Dopamine (DA)…' that starts on line 16 of page 4.

   Answer: *This statement refers still to the Cools et al. (2011) paper, which we clarified by adding the reference again on page 4.*

2. When discussing Figner et al (2010), please denote TMS to mean transcranial magnetic stimulation. Most readers will already know, but perhaps a few may not. Please also note whether the TMS administered was of low or high frequency (they can have opposing effects) and whether it was one or multiple sessions.

   Answer: *We follow the reviewer's advice and gave the requested details about the study on page 5.*

3. Page 6, line 25. You have a typo – 'Previous studies suggest that S-allele carrier(s) have…'. Please fix.

   Answer: *We thank the reviewer for pointing this out and changed it accordingly.*

4. I suggest referring to the control condition as just that, rather than 'an intermediate control condition', which seems less correct (page 7, line 12).

   Answer: *We agree with the reviewer and changed it as suggested.*

5. Please define what you mean by 'a visus of less than 0.8' on page 7.

   Answer: *We agree that this may be confusing and changed "visus" more precisely to visual acuity (now on page 8)*

6. Page 7/8: Inclusion/Exclusion criteria. Please explain whether you allowed illicit drug use, and when the cut-off for this was if so. Surely no drugs were allowed to be in the system on the day of testing? If so, this is a major issue. If you did not test for illicit drug use, then this should be noted as a limitation.

   Answer: *We thank the reviewer for this comment and clarified that we tested for illicit drug use with a urine test during the first session, which would have led to exclusion from the study. We also tested breath-alcohol at the beginning of each session. We added the following sentence: "In the first session, a urine test was conducted to test for illicit drug use (Kombi/DOA10-Schnelltest, MAHSAN Diagnostika GmbH, Reinbek, Germany) and in every session breath-alcohol was measured (Alcotest 6510, Dräger, Lübeck, Germany)".*

7. The results section is a little difficult to follow because you are reporting results from 2 separate studies (transporter and behaviour, and depletion, fMRI and behaviour). I recommend labelling the two datasets more clearly – perhaps dataset 1 and dataset 2, or study 1 and study 2 could suffice.

   Answer: *We agree with the reviewer and referred to the studies in the results section as genetic and pharmacological study, respectively (p. 20).*

Reviewer #2: Thank you for asking me to review this interesting manuscript on the potential role of the 5-HT system on intertemporal choice. The study is an interesting addition to the literature on the topic. The manuscript is well written.

Answer: *We cordially thank the reviewer for his/her positive reception of our study and manuscript.*

I note a few minor typographical errors or stylistic comments.

- I think on page 5 line 17 the word "consent" is written where "consensus" might be better.

  Answer: *We agree with the reviewer that consensus is the appropriate term to use. We changed it accordingly.*

- Page 7 line 48, I am unsure if the use of "visus of less than 0.8" is commonplace outside of Germany. Perhaps a simple statement of good enough visual acuity (corrected or uncorrected) would be more widely understood.

  Answer: *We thank the reviewer for the suggestion and changed "visus" more precisely to visual acuity (now on page 8)*

- Page 11, line 11 "carbon hydrates" should read "carbohydrates".

  Answer: *We agree with the reviewer and changed the word accordingly.*

Reviewer #3: This is a very thoroughly conducted and well-designed study of the effects of serotonin and 5-HTTLPR on delay discounting and associated BOLD responses. The topic is very interesting and although results were contrary to the authors' hypothesis the null findings of this study are important and informative to the scientific community. I congratulate the authors for their research. I have no comments/suggestions to improve the quality of this paper.

Answer: *We are very happy about the strong enthusiasm of the reviewer regarding our study and thank him/her very much for the commendations!*

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Auf der privaten Seite gibt es gleichermaßen viele Menschen, die mich auf diesem Weg begleitet haben und ein intrinsisches Bedürfnis hatten, Zeit mit mir außerhalb der Arbeit zu verbringen, wofür ihnen allen mein Dank gewiss ist. Das betrifft Schul- und Studienfreunde, und gute Bekannte die mir mit Rat, Spaß, Brot und Bier zur Seite standen, sowie Kollegen, mit denen ich mich mittlerweile in Freundschaft verbunden fühle, insbesondere Edgar Arrua Vares, Yacila I. Deza Araujo, Shakoor Pooseh, Ying Lee und Johannes Petzold.

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Erklärung

Diese Dissertation wurde in der Zeit vom 01/2013 bis 01/2020 unter der Betreuung von Prof. Dr. med. Michael N. Smolka im Forschungsbereich Systemische Neurowissenschaften, Klinik und Poliklinik für Psychiatrie und Psychotherapie, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden angefertigt. Es haben keine früheren erfolglosen Promotionsverfahren stattgefunden. Die Promotionsordnung der Fakultät für Mathematik und Naturwissenschaften vom 23. Februar 2011 erkenne ich an.

Versicherung

Hiermit versichere ich, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht. Die Arbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

06.02.2020

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Philipp Neukam